ABSTRACT

PPP1R2 COORDINATES KINASE AND PHOSPHATASE ACTIVITY TO REGULATE THE CENTROSOME AND MIDBODY

by

Alan-Michael Jay Bresch

May, 2021

Director of Dissertation: Ann O. Sperry, PhD

Major Department: Anatomy and Cell Biology

Centrosomes are the primary microtubule organizing centers of the cell and prepare the cell for division by establishing the bipolar spindle during mitosis. A balance of kinase and phosphatase activity regulates centrosome number and mitotic spindle function. PPP1R2 is a negative regulator of Protein Phosphatase 1, PP1, and an activator of Aurora A Kinase, AURKA. Both PP1 and AURKA play critical roles in centrosome regulation, however PPP1R2's role at the centrosome has not been examined. Given that PPP1R2 interacts with PP1 and AURKA, critical regulators of the centrosome, I hypothesized that PPP1R2 is a key regulator of the centrosome cycle through its interaction with AURKA and PP1. I tested this hypothesis through an overexpression model using PPP1R2, PPP1R2 truncation mutants, PPP1R2 phosphomutants, AURKA, and PP1. PPP1R2, AURKA, and PP1 overexpression resulted in both supernumerary centrosomes and γ-tubulin mislocalization. In addition, PPP1R2 truncation mutant overexpression resulted in similar effects at the centrosome. Only PPP1R2 phosphomimetic mutant overexpression resulted in increased centrosome number. PPP1R2 and PPP1R2 mutant overexpression also resulted in disruption of midbody architecture as well an increase in polyploidy. PPP1R2 truncation mutant overexpression as well as PPP1R2 phosphomimetic, PPP1R2E significantly decreased PP1 midbody

localization. PPP1R2's regulation of PP1 midbody recruitment further supported PPP1R2's role in midbody regulation. Lastly, PPP1R2 overexpression reduced phosphorylation levels of Plk1, AURKA, and PP1 at the centrosome compared to the control. Overall, I found that PPP1R2 does coordinate PP1 and AURKA activities to regulate the centrosome protein recruitment as well as centrosome number through midbody architecture maintenance. In addition, PPP1R2 regulated microtubule nucleation from the centrosome and this regulation was dependent on PPP1R2's phosphorylation state. This dissertation demonstrates that PPP1R2 is a critical regulator of centrosome as well as midbody structure and function through AURKA and PP1 activity coordination.

PPP1R2 COORDINATES KINASE AND PHOSPHATASE ACTIVITY TO REGULATE THE CENTROSOME AND MIDBODY

A Dissertation

Presented To

The Faculty of the Department of Anatomy and Cell Biology

East Carolina University

In Partial Fulfillment

Of the Requirements for the Degree

Doctor of Philosophy in Anatomy and Cell Biology

by

Alan-Michael Bresch

May, 2021

PPP1R2 COORDINATES KINASE AND PHOSPHATASE ACTIVITY
TO REGULATE THE CENTROSOME AND MIDBODY

by

Alan-Michael Bresch

APPROVED BY:

DIRECTOR OF

DISSERTATION

COMMITTEE MEMBER: Ann O. Sperry, PhD

COMMITTEE MEMBER: Jitka Virag, PhD

Chris Geyer, PhD

COMMITTEE MEMBER:

Jeffrey Eells, PhD

CHAIR OF THE DEPARTMENT

OF Gross Anatomy and Cell Biology

Peter Koch, PhD

DEAN OF THE

GRADUATE SCHOOL:

Paul J. Gemperline, PhD

DEDICATION

To my parents Ricky and Sharon Bresch without whom this would be impossible.

To my little brother Andrew Bresch who was there to support me in hard times.

To my best friends Logan Kelly Davis, Hayden Huggins, Cody Stuckensneider, and Nicholas Serra, who completed the bulwark of my support group.

ACKNOWLEDGEMENTS

I would first humbly thank my God for providing me the necessary resources and ability to complete this project. He has blessed me richly and deserves full credit for this project's completion. I formally extend my appreciation to my parents Ricky and Sharon Bresch. Their love, patience, and understanding has refilled my soul on many occasions and has given me the courage and fortitude to persevere. I thank my brother for his quiet support during the difficult seasons. He made his presence known and offered his attention whenever I asked. I lastly thank my grandmother Clara Russell whose enthusiasm and wit infected me with a desire to be the best I could become. Her support was always felt, even in times of her sickness.

I would like to thank Rong Wang for her expert guidance and instruction. Her experience developed my technical skills and her advice developed me as a person. She was a wonderful coworker to be alongside. Her support and kindness allowed me to stretch my wings in the laboratory and learn how to be an effective scientist.

I would like to thank my closest friends Logan Davis, Cody Stuckensneider, Nick Serra, and Hayden Huggins for their attentive care and the great memories. The times we have shared have been invaluable to me and have helped to shape me into a better person. I will forever cherish their friendship.

I would like to extend appreciation to the Department of Anatomy and Cell Biology for the education, academic development, and opportunities provided that honed my lecturing skills.

I also appreciate the Department for providing an environment that has fostered great personal growth. It has also provided great mentors who have advised me through times of difficulty.

Finally, I would like to show appreciation towards my mentor Dr. Ann O. Sperry. Her diligence to accuracy and precision as well as profound scientific knowledge and understanding was a guiding light. Her advice and countless hours of editing as well as constructive criticism shaped me into the scientist I am today. I was able to grow and learn abundantly under her tutelage for which I am grateful.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

γ-TURC	γ-tubulin ring complex
ANOVA	Analysis of Variance
ARPE-19	Human Retinal Pigmented Epithelial
AURKA	Aurora A kinase
AURKB	Aurora B kinase
BSA	Bovine Serum Albumin
cDNA	Copy deoxyribonucleic acid
Cep55	Centrosome related protein 55 kDa
Cep192	Centrosome related protein 192 kDa
Cep215	Centrosome related protein 215 kDa
Cep250	Centrosome related protein 250 kDa
DAPI	4’,6-diamidino-2-phenylindole
DMEM-F12	Dulbecco’s modified eagle medium/nutrient mixture F-12
DNA	Deoxyribonucleic acid

EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
ESCRT I, II, & III	Endosomal sorting complex I, II, & III
GFP	Green Fluorescent Protein
GSK3	Glycogen synthase kinase 3
hGCP	γ-tubulin complex component
HRP	Horseradish peroxidase
iASPP	i-Apoptosis-stimulating protein of p53
kDa	Kilodalton
MTOC	Microtubule organizing center
MZT1	Mitotic spindle organizing protein 1
NEDD1	Neural precursor cell expressed, developmentally down-regulated 1
Nek-2	NIMA-related kinase 2
NIMA	Never in mitosis gene-A
PCM	Pericentriolar matrix

PCNT	Pericentrin
Plk-1	Polo-like Kinase 1
Plk-4	Polo-like Kinase 4
PMSF	Phenylmethylsulfonyl fluoride
PP1	Protein Phosphatase 1
pR2	Phosphorylated R2
PVDF	Polyvinylidene difluoride
R2ΔC	R2 C-terminal truncation mutation
R2ΔN	R2 N-terminal truncation mutation
R2A	R2 Threonine residue number 73 Alanine mutation
R2E	R2 Threonine residue number 73 Glutamic Acid mutation
SAS-5	Spindle assembly abnormal protein 5 homologue
SAS-6	Spindle assembly abnormal protein 6 homologue
SDS	Sodium Dodecyl Sulfide
SDS-PAGE	SDS-Polyacrylamide gel electrophoresis

TBST	Tris-Buffered Saline and Triton X-100
Thr72	Threonine residue number 72
Thr73	Threonine residue number 73
Thr288	Threonine residue number 288
TPX2	Targeting protein for Xklp2
TPXL-1	Targeting protein for Xklp2 homologue 1
TTBS	Tris-Buffered Saline and Tween 20
XMAP215	Microtubule associated protein 215 kDa
ZYG-1	Zygote defective protein 1

CHAPTER I: INTRODUCTION

A. Rationale

Cancer is a significant global health problem and is the second leading cause of death in the United States (Siegel, R. L. et al., 2020). General cancer treatments are nonspecific and costly. Common treatments for cancer such as radiation and chemotherapy have damaging effects on tissues, and thus cause a range of negative side effects. Biologics have also been developed that specifically target tumor tissue. This approach is termed cancer immunotherapy and uses tumor-specific antigens, lymphocytes, and T-cells to illicit an immune response against a patient's tumor. It remains a costly treatment and has been shown to be ineffective against more advanced cancer diseases as well as large tumor masses. None of the abovementioned treatments have been able to eradicate cancer. This presents a profound burden on the health care system and reduces the quality of life of cancer patients.

Tumorigenesis, the transformation of normal cells into abnormally functioning cancer cells, occurs through dysregulation of cell function including cell cycle control. The centrosome is closely regulated alongside the cell cycle through overlapping enzymes and establishes the bipolar spindle during mitosis. There is increasing evidence that centrosome amplification correlates with tumorigenesis because tumor cells have more centrosomes than their untransformed counterparts (Levine, M. S., 2017; Rivera-Rivera, Y., & Saavedra, H. I., 2016). To understand the specific mechanisms linking centrosome dysregulation to cancer, it is necessary to understand normal centrosome function.

B. Centrosome Structure and Centrosome Regulation

Centrosomes are nonmembrane-bound organelles consisting of perpendicularly oriented centrioles, cylindrical structures comprised of highly acetylated and stable microtubules, surrounded by a protein scaffold called the pericentriolar matrix (PCM) The centrosome's nonmembrane-bound structure allows it to directly interface with the surrounding cytoplasm. It does so by assembling complex microtubule networks which act as a spatial organizing system within the cell

Disruption of centrosome structure and function leads to a variety of cellular defects, including abnormal chromosome segregation and tumorigenesis (Godinho & Pellman, 2014). by centrosome amplification establishing supernumerary, more than 2, centrosomes in a cell. Multiple centrosomes increase the incidence of transient multipolar spindles which results in abnormal chromosome attachment and abnormal chromosome segregation during cell division. Missegregated and lagging chromosomes lead to aneuploidy correlated to tumorigenesis (Godinho & Pellman, 2014).

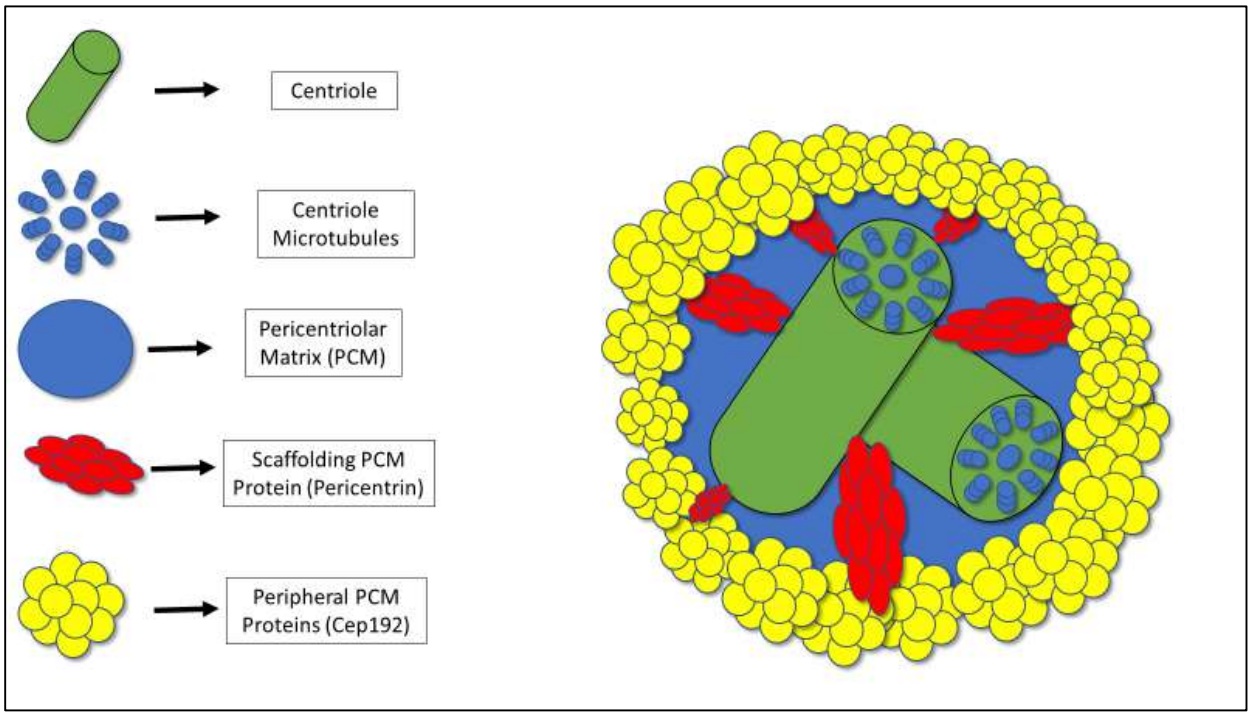
Centriole
Centriole Microtubules
Pericentriolar Matrix (PCM)
Scaffolding PCM Protein (Pericentrin)
Peripheral PCM Proteins (Cep192)

Figure 1.1. A schematic of centrosome structure. Perpendicular centrioles are indicated in green, the pericentriolar matrix in blue, and the two primary types of PCM proteins are indicated in red and yellow).

Direct recruitment of proteins to the PCM remodels centrosome structure throughout the cell cycle. Centrosome assembly, the process of recruiting critical proteins to its structure, shares overlapping regulation with important cell cycle events during each phase of the cell cycle (Breslow, D. K., & Holland, A. J., 2019; Conduit, P. T. et al.,). The centrosome is assembled during the centrosome cycle, which includes centriole disengagement, centrosome duplication, centrosome maturation, centrosome separation, and mitotic spindle assembly. Centrosome disengagement occurs during G_1 phase, when the centrioles of the centrosome inherited from the previous dividing cell separate. Centrosome duplication then begins during S phase, when the disengaged centriole nucleates a procentriole; this process occurs within the same cell cycle phase as chromosome duplication. Both duplication events are required to prepare the cell for division and eventual symmetric chromosome segregation. The newly nucleated procentrioles, renamed the daughter centrioles, elongate and recruit PCM through a process called centrosome maturation. Centrosome maturation prepares fully matured centrosomes for mitotic spindle assembly and occurs duringthe transition from G_2 phase to mitosis. Once fully mature, the centrosomes separate and migrate to opposite sides of the cell and assemble the bipolar mitotic spindle. Each of these events is necessary to produce duplicated centrosomes required for mitotic spindle assembly during mitosis. Dysregulation of these events can lead to tetraploidization, aneuploidy, asymmetric inheritance of centrosomes or chromosomes, mitotic spindle defects, and delayed cell division with mitotic failure (Schatten, H., & Sun, Q. Y., 2018).

The assembly and structural integrity of the centrosome is regulated by phosphorylation which alters both protein structure as well as protein-protein affinity and is responsible for regulating centrosome protein recruitment (Fujita, H. Y., 2016). The central mechanism for centrosome cycle regulation is the positive relationship between protein recruitment and phosphorylation. Protein phosphorylation couples the centrosome cycle to the cell cycle and is critical to prepare the cell for division (Sluder, G., 2005; Wang, G., Jiang, Q., & Zhang, C. 2014; Yaguchi, K. et al., 2018). Specifically, coordination of phosphorylation at the centrosome is essential for both cell cycle progression as well as cell division.

The centrosome is a central microtubule organizing center leading to its unique role within cells as a central platform for organelle and vesicle trafficking, cell signaling, cell motility, and cell division (Cheng, H. W. et al., 2019; Conduit, P. T., Wainman, A., & Raff, J. W., 2015; Krämer, A. Lukas, J. Bartek, J., 2004; Schatten, H., 2008). Centrosomes are also signaling cascade centers as the activity level of critical cell cycle enzymes are regulated at the PCM and vesicle trafficking is associated with the MTOC (Krämer, A., Lukas, J., & Bartek, J., 2004; Schatten, 2008). Cell signaling complexes travel along centrosome-organized microtubules, which provide a physical pathway for cell signaling complex activity and interaction. There are several critical cell cycle signaling pathways regulated in this manner, linking the centrosome cycle to the cell cycle (Schatten, H., 2008) Several of these proteins are necessary to maintain both centrosome maturation and cell cycle checkpoint regulation (Fujita, H. Y., 2016; Schatten, H., 2008). Examples include pericentrin, Cep192, Plk1, Aurora A Kinase, and PP1, which are necessary to both prepare the cell for division as well as drive cell cycle progression (Gomez-Ferraria, M. A. et al., 2007; Joukov, V., Walter, J. C., & De Nicolo A., 2014; Joukov, V., & De Nicolo, A., 2018; Lee, K., & Rhee, K., 2011; Mattison, C. P., & Winey, M., 2006; Meraldi, P., & Nigg, E. A., 2002). The activities of these proteins are regulated through posttranslational modifications, predominantly serine/threonine phosphorylation (Breslow, D. K., & Holland, A. J., 2019; Conduit P. T. et al., 2014; Fujita, H. Y., 2016; Nigg, E. A., & Stearns, T., 2011).

B1. Centrosome Maturation: γ-tubulin Ring Complex (γ-TURC) Assembly and Recruitment

The centrosome is largely disassembled following cell division and PCM protein phosphorylation drives recruitment of critical microtubule assembly complexes resulting in stepwise centrosome reassembly (Gomez-Ferreria, M. A. et al., 2012; Joukov, V., Walter, J. C., & De Nicolo, A., 2014; Kim, J., Lee, K. & Rhee, K., 2015; Lee, K. & Rhee, K., 2011; Lin, T. C. et al., 2014; Pinyol, R., Scrofani, J., & Vernos, I., 2013). Beginning at G_1 phase, scaffolding proteins including Cep250 and pericentrin are recruited to the centrosome to begin the assembly of the PCM, a matrix of proteins that forms the periphery of the centrosome surrounding the centrosome's centrioles (Magescas, J., Zonka, J. C., & Feldman, J., 2019; Palazzo, R. E. et al. 2000). The PCM is fully assembled during late S phase and G_2 phase in a process called

centrosome maturation (Fujita, H. Y., 2016; Palazzo, R. E., et al., 2000; Wang, G. et al., 2014). During centrosome maturation, Cep192 interacts with pericentrin to recruit critical kinases including Polo-like Kinase 1 (Plk1) and Aurora A Kinase (AURKA) as well as an essential phosphatase Protein Phosphatase 1 (PP1) to the centrosome (Joukov, V. et al., 2014; Lee, K., & Rhee, K., 2011; Nasa, I., 2017). AURKA and Plk1 recriutment to the PCM results in a net increase in phosphorylation at the centrosome, which causes hyperphosphorylation of pericentrin and Cep192 as well as phosphorylation of Nedd1 (Gomez-Ferreria, M. A. et al., Joukov, V. et al, 2014, 2012; Haren, L. et al., 2006; Haren, L. et al., 2009; Lee, K. & Rhee, K., 2011; Manning, J. A., Shalini, S., Risk, J. M., Day, C. L., & Kumar, S., 2010; Zhang, X. et al., 2009).

Microtubule nucleation at the centrosome is facilitated by γ-tubulin ring complexes (γ-TURC) which form a stable template for microtubule negative end polymerization (Liu, P. et al., 2020; Raynaud-Messina & Merdes, 2007; Tovey & Conduit, 2018). Nedd1 is a scaffolding protein for γ-tubulin ring complexes (γ-TURC), and it recruits these complexes to the centrosome following the shift in centrosome phosphorylation caused by changes in AURKA and Plk1 activity (Gomez-Ferreria, M. A. et al., 2012; Haren, L. et al., 2006; Manning, J. A. et al., 2010; Zhang, X. et al., 2009). α-tubulin and β-tubulin form microtubules, which on their own are unstable in the cytoplasm, with the exception of neural microtubules in the axon (Desai, A., & Mitchison, T. J., 1997; Gireesh, K. K. et al., 2018; Margolis, R. L., & Wilson, L., 1998; Vemu, A. et al., 2018; Wade, R. H., 2007; Waterman-Storer, C. M., & Salmon, E. D, 1997). γ-tubulin complexes serve as an anchor for microtubule negative ends, increasing structural stability by forming a ring of γ-tubulin that stably associates with α and β-tubulin dimers. The centrosome is considered fully mature when PMC assembly is complete. Following complete centrosome maturation, the recruitment of PCM proteins is regulated by phosphorylation and is critical for the assembly of the mitotic spindle. The structure of the pericentriolar matrix changes dramatically between interphase and mitosis due to an increase in phosphorylation of PCM scaffolding proteins including pericentrin and Cep192. The PCM transitions from a highly regulated toroidal shape to a larger and irregular structure. (Gomez-Ferreria, M.

A., & Sharp, D. J., 2008; Larsson, V. J. et al., 2018; Lüders, J., 2012; Prosser, S. L., & Pelletier, L., 2017; Woodruff, J. B. et al., 2014).

C. Centrosome Function

C1. Assembly of the Mitotic Spindle

As an integral part of the cell's microtubule cytoskeleton, centrosomes are responsible for organizing the mitotic spindle. Proper regulation of centrosome dynamics is required for mitotic spindle assembly and chromosome alignment in mitosis (Gomez-Ferrerria, M. A. et al., 2012; Greenan, G. et al., 2010; Hoffman, I., 2020; Keller, L. C., Wemmer, K. A., & Marshall, W. F., 2010; Meraldi, P., 2016; Pinyol, R., 2013). The turnover rate of pericentriolar matrix components at the centrosome, specifically pericentrin and γ-TURC, is dynamic throughout the cell cycle (Khodjakov, A., & Rider, C. L., 1999; Lüders, J., 2012). Suppressed protein recruitment alters microtubule nucleation at the centrosome (Jeffery, J. M. et al., 2013; Lee, S. & Rhee, K. 2010). Altogether, phosphorylation is essential to maintain centrosome protein recruitment and as an extension to regulate microtubule nucleation and the function of the mitotic spindle (Gomez-Ferreria, M. A. et al., 2012; Guo, L. et al., 2019; Joukov, V., Walter, J. C., De Nicolo, A., 2014; Lee, K., & Rhee, K. 2011; Lu, M. S., & Johnston, C. A., 2013; Miyamoto, T. et al., 2017; Mukherjee, M. et al., 2018; Pinyol, R. Scrofani, J., & Vernos, I., 2013;).

C2. Centrosome Cycle and Cell Cycle Coupling

Multiple events during the centrosome cycle are coupled to the cell cycle through centrosome structure and function (Adon, A. M. et al., 2010; Arlot-Bonnemains, Y., & Prigent, C., 2002; Keck, J. M. et al., 2011; Lutz, W. et al., 2001; Mbom, B. C., Nelson, W. J., & Barth, A., 2013; Patzke, S. et al., 2005; Srsen, V., Gnadt, N., Dammermann, A., & Merdes, A., 2006; Vandré, D. D., Feng, Y., & Ding, M., 2000). The centrosome is coupled to the cell cycle through both direct structural regulation (Kim, S. & Tsiokas, L., 2011), such as centrosome-mediated ciliogenesis and cell cycle progression from G_1 to S phase, and enzymatic coordination of phosphorylation levels between substrates involved with the centrosome and cell

cycle (Adon, A. M. et al., 2010; Keck, J. M. et al., 2011; Vandré, D. D., et al., 2000). Most relevant to this dissertation is the overlap of both cell cycle and centrosome targets shared between PP1, Plk1, and AURKA (Cowley, D. O. et al., 2009; Joukov, V., et al., 2014; Joukov, V., & De Nicolo, A., 2018; Kim, J., Lee, K., & Rhee, K., 2015; Lee, K., & Rhee, K., 2011; Nasa, I., 2017). These three enzymes interact with substrates shared at critical cell and centrosome cycle junctures during G_2 phase and mitosis (Joukov, V. et al., 2014; Joukov, V., & De Nicolo, A., 2018; Lee, K., & Rhee, K., 2011; Nasa, I., 2017). Overall, the precise regulation of both the cell and centrosome cycles prepares the cell for radical cytoskeletal changes during mitosis that are essential for cell division. Without proper coordination of the cell and centrosome cycles, neither the centrosome nor the cytoskeleton are able to orchestrate proper cell division, resulting in disrupted daughter cells (Gemble S. et al., 2019; Hinchcliffe, E. H., 2014; Lingle, W. L., Lukasiewicz, K., & Salisbury, J. L., 2005; Vora, S., & Phillips, B. T., 2015).

D. Midbody Structure

D1. Midbody Assembly

One critical cell structure change during mitosis is midbody assembly from the mitotic spindle and contractile ring. Midbody assembly and maintenance begins in anaphase and ends with cell division through a process called cytokinesis in which the cytoplasm of two dividing daughter cells is completely separated (Antanavičiūtė, I. et al., 2018; Green, R. A. et al., 2013; Gulluni, F., Martini, M., & Hirsch, E., 2017). Midbody structure includes a remnant of the mitotic spindle, the central spindle, as well as an actomyosin structure called the contractile ring which forms its center. The midbody interacts with the surrounding cell membrane to form an intercellular bridge that is responsible for maintaining a cytoplasmic connection between two daughter cells until cell division is complete. Cytokinesis and later abscission, cellular fission separating two daughter cells, are dependent upon the joint regulation of mitotic and centrosome regulators described previously, as their phosphorylation results in the timely recruitment of complexes necessary for

both midbody formation and function (Adriaans, I. E. et al., 2019; Bastos, R. N., & Barr, F. A., 2010; Bhowmick, R. et al., 2019; Fabbro, M. et al., 2005; Gao, K. et al., 2018; Li, Q. et al., 2014; Uehara, R. et al., 2013) .

Phosphorylation as well as cytoskeletal scaffolding proteins also play a key a role in recruiting necessary protein complexes to facilitate the radical shift in the cell's cytoskeleton necessary for midbody establishment (Antanavičiūtė, I. et al., 2018; D'Avino, P. P., & Capalbo, L., 2016; Gao, K., 2018; Gao, K. et al., 2018; Green, R. A. et al., 2013; Hu, C. K., Coughlin, M., & Mitchison, T. J., 2012; Pike, T. et al., 2016; Sun, S. et al., 2016). A net increase in dephosphorylation at the mitotic spindle provides the initial signal to coordinately disassemble the spindle and transition microtubule structure into the midbody's central spindle.

D2. Disassembly of Mitotic Spindle and Assembly of Midzone

The transition of the mitotic spindle into the midbody is an elegant restructuring of both the microtubule and actomyosin cytoskeleton. This transition begins during cytokinesis during early anaphase to late telophase. Cytokinesis begins as the actomyosin contractile fibers constrict the cell membrane into a cleavage furrow, and condensed nuclei are enclosed within membranes that will form the future daughter cell nuclei. A midzone forms between the new daughter cell nuclei as the cleavage furrow develops (Pamula, M. C. et al., 2019).This midzone consists of interpolar microtubules which provide a scaffold for the motor proteins necessary for the migration of the newly condensed nuclei to opposite poles of the dividing cell (Hannabuss, J. et al., 2019). During late anaphase, these interpolar microtubules are then bundled into antiparallel arrays (Hannabuss, J. et al., 2019; Pamula, M. C. et al., 2019). This remodeling of the midzone occurs simultaneously with actomyosin contractile ring formation around the equator of the cell. The contractile ring likewise begins to establish the cleavage furrow. The cleavage furrow ingresses in order to separate the membranes of the newly formed daughter cells and is most prominent at the equatorial cortex during anaphase (D'Avino, P. P., Savoian, M. S., & Glover, D. M., 2005). Cleavage furrow

formation must occur at a highly regulated time and position in order to ensure proper chromosome segregation (Liu, Z. & Weiner, O. D., 2016) (Figure 1B)

D3. Formation of Midbody and Abscission Machinery

The midbody is assembled from microtubules, actomyosin, and the cell membrane which allows the recruitment of machinery necessary for completing cell division. During telophase, the contractile ring fully constricts the cell equator and interacts with the bundled microtubules originating from the midzone. Together, the constricted cell membrane, contractile ring, and bundled midzone form the midbody. At this point, the contractile ring is renamed the midbody ring and the bundled midzone becomes the central spindle (Bassi, Z. I., Audusseau, M., Riparbelli, D., Callaini, G., & D'Avino, P. P. 2013; Courthéoux, T. et al., 2019; El-Amine, N., Carim, S. C., Wernike, D., & Hickson, G. R., 2019). The interaction between the microtubules, cell membrane, and actomyosin components of the midbody is through large scaffolding complexes consisting of centralspindlin, septins, and centrosome-related proteins including Cep55.

The dividing cell begins to organize abscission machinery necessary for the final step of cell division through phosphorylation and protein recruitment. Abscission is the fission of midbody as well as the cellular membrane. Abscission marks the complete separation of both cytoplasm and membrane between the daughter cells (Gulluni, F. et al., 2017; Nähse, V. et al., 2017; Patharkar, O. R., & Walker, J. C., 2018; Steigemann, P., & Gerlich, D. W., 2009). Essential proteins assemble abscission machinery through a series of phosphorylation events which fully separate the daughter cells (Green, R. A. et al., 2013; Gulluni, F., et al., 2017; Lie-Jensen, A., et al., 2019; Nähse, V. et al., 2017; Pike, T. et al., 2016; Sun, S. et al., 2016). The abscission machinery consists of multimeric complexes comprised of ESCRT I-III and ATPases (Addi, C., Bai, J., & Echard, A., 2018; Christ, L. et al., 2016; Christ, L. et al., 2017; Guizetti, J., & Gerlich, D. W., 2010; Henne, W. M., Buchkovich, N. J., & Emr, S. D., 2011). During late telophase, abscission occurs when the fully assembled abscission machinery remodels the cell membrane and cleaves the midbody to separate the daughter cells.

E. Midbody Function

During cytokinesis, the midbody is the central structure that acts as a scaffold for the abscission machinery necessary for the completion of cell division (Antanavičiūtė, I. et al., 2018; D'Avino, P. P., & Capalbo, L., 2016; Green, R. A., et al., 2013; Hu, C. K., et al., 2012; Lie-Jensen, A., et al., 2019; Steigemann, P., & Gerlich, D. W. 2009). The midbody is the interface between the actomyosin contractile ring, a ring of F-actin and myosin-2 that generates the force necessary for cell separation, and the central spindle. Together, these structures form an intercellular bridge between two daughter cells prior to abscission. (Gulluni, F., Martini, M., & Hirsch, E., 2017; Nähse et al., 2017; Patharkar, O. R., & Walker, J. C., 2018; Steigemann, P., and Gerlich, D. W., 2009). It is during cytokinesis that phosphorylation of microtubule and actomyosin proteins regulates the timely recruitment of protein complexes that assemble the contractile ring, central spindle, midbody, and finally the abscission machinery (Gao, K. et al., 2018; Jungas, T. et al., 2016; Pike, T. et al., 2016; Steigemann, P. et al., 2009; Sun, S. et al., 2016). Following the completion of cell division by abscission, both the centrosomes and chromosomes are segregated equally between the daughter cells. Misregulation of protein phosphorylation can result in defects in this process leading to an improper number of both chromosomes and centrosomes in resulting daughter cells.

E1. Phosphorylation Regulation of Midbody

Phosphorylation also regulates the length of the midbody's central spindle. This is achieved by a balance of phosphatase and kinase activities. Phosphorylation of Kif4, a negative regulator of plus-end microtubule dynamics, is phosphorylated by multiple kinases resulting in coordinated control of midbody length (Li, Q. R. et al., 2017; Uehara, R. et al., 2013). The length of the midbody is highly conserved from cell to cell and is maintained through motor proteins like Kif4. Altered phosphorylation levels of midbody affiliated motor proteins can disrupt midbody structure and lead to defects in cytokinesis including temporal delays and abnormal protein complex formation (Li, Q. R. et al., 2018; Nunes B. et al., 2013; Uehara, R. T. et al., 2013).

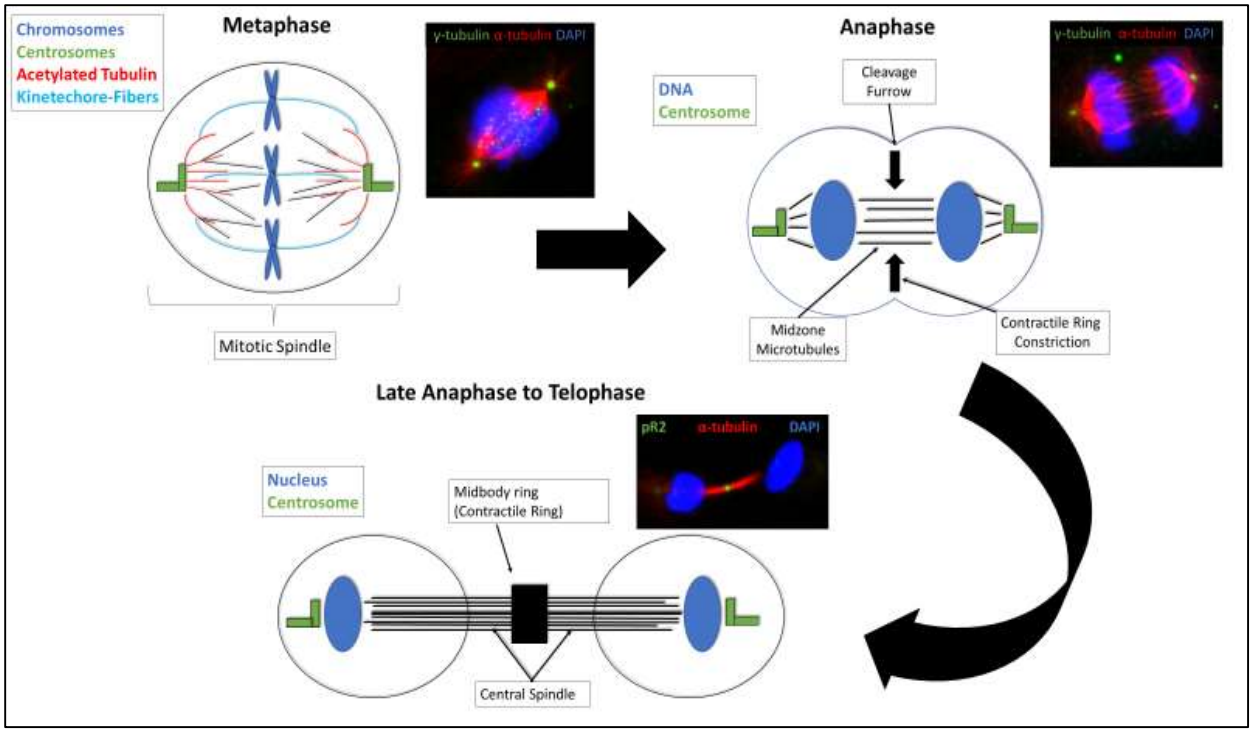
Chromosomes
Centrosomes
Acetylated Tubulin
Kinetechore-Fibers
Metaphase
γ-tubulin α-tubulin DAPI
Mitotic Spindle
Anaphase
DNA
Centrosome
Cleavage Furrow
γ-tubulin α-tubulin DAPI
Midzone Microtubules
Contractile Ring Constriction
Late Anaphase to Telophase
pR2 α-tubulin DAPI
Nucleus
Centrosome
Midbody ring (Contractile Ring)
Central Spindle

Figure 1.2. Schematic of cytoskeletal changes taking place from metaphase to telophase. This diagram includes different microtubule arrangements during metaphase, the simultaneous formation of cleavage furrow and midzone, and the interaction of microtubules,

F. Kinases and Phosphatases that Maintain Centrosome Structure and Function

F1. AURKA

AURKA belongs to a family of three Aurora kinases (Carmena, M., & Earnshaw, W. C., 2003) that regulate the centrosome during centrosome maturation and cell division. During late S phase, AURKA binds to Cep192 and is recruited to the centrosome as the cell transitions into G_2 phase (Joukov, V. et al., 2014). AURKA's primary function during G_2 phase is to activate Plk1 and phosphorylate Cep192 during centrosome maturation which results in the recruitment of γ-TURC (Carmena, M., & Earnshaw, W. C., 2003; Joukov, V. et al., 2014). γ-TURC, anchored by pericentrin and Cep192, is responsible for microtubule nucleation from the centrosome and has a role in organizing the mitotic spindle during mitosis (Zimmerman, W. C., Sillibourne, J., Rosa, J., & Doxsey, S. J., 2004). An important interactor of AURKA, TPX2, is released from the nucleus following nuclear membrane disassembly. AURKA then binds to TPX2, which targets AURKA to microtubules of the mitotic spindle (Bayliss, R. et al., 2003; Bayliss, R. et al., 2004; Zorba, A. et al., 2014). Centrosome-bound AURKA maintains the stability of the spindle by recruiting γ-TURC to the centrosome, and TPX2-bound AURKA phosphorylates microtubules and microtubule complexes (Cowley, D. O. et al., 2009; Kufer, T. A. et al., 2003; Magnaghi-Jaulin, L. et al., 2019). AURKA phosphorylates mitotic spindle proteins, including the motor protein kif15 and mitotic spindle organizer ASAP (Naso, F. D. et al., 2020; van Heesbeen, R. G. et al., 2017; Venoux, M. et al., 2008) to remodel the mitotic spindle's structure and enable proper capture and alignment of chromosomes at metaphase. GM130 of the Golgi apparatus positively regulates formation of TPX-2-bound AURKA complexes responsible for maintaining local microtubule nucleation. GM130, a Golgi matrix protein, sequesters importin α which releases TPX-2 to bind AURKA. GM130 then captures newly nucleated microtubules and links the Golgi membrane to the mitotic spindle (Wei, J. H. et al., 2015). It is not known whether AURKA plays a direct role in regulating the midbody following metaphase. AURKA has been shown to play an active role in clearing contractile elements at the cell cortex following the disassembly of the mitotic spindle. The clearing of these contractile elements at the cell's cortex is necessary for restricting

them to the cell's equator (Mangal, S. J., 2018). In addition, AURKA establishes a phosphorylation gradient across the midzone in concert with Aurora B kinase (AURKB) (Ye, A. A., Torabi, J., & Maresca, T. J., 2016; Fuller, B. G., Lampson, M. A., Foley, E. A., Rosasco-Nitcher, S., Le, K. V., Tobelmann, P., Brautigan, D. L., Stukenberg, P. T., Kapoor, T. M., 2008). The established gradient is involved in both contractile ring positioning as well as successful cytokinesis of two daughter cells (Ye, A. A., Torabi, J., & Maresca, T. J., 2016). The phosphorylation gradient established by both AURKA and AURKB were demonstrated in multiple cell lines including both insect (S2, S3) and mammalian (HeLa, Du145, DLD21). It remains uncertain whether AURKA and AURKB establish phosphorylation gradients similarly in untransformed mammalian cells. However, this dissertation's findings suggest that AURKA may regulate the midbody directly in ARPE cells, an untransformed retinal epithelial cell line.

F2. Cytokinesis

Two distinct mechanisms regulate AURKA's activity. First, AURKA autophosphorylates at Thr288, which is located in its activation loop (Bertolin, G. et al., 2016; Ohashi, S. et al., 2006) to activate and increase its activity in all cells. AURKA activation by autophosphorylation is predominantly affiliated with AURKA pools at the centrosome. Second, AURKA's interaction with its subunit TPX2 protects AURKA's activation loop from dephosphorylation through a conformational change in AURKA. This conformation change prolongs activation of AURKA by preventing PP1 access to phospho-residues located in AURKA's activation loop. AURKA autophosphorylation at the centrosome regulates the recruitment of proteins such as pericentrin and Nedd1. (Bayliss, R. et al., 2003; Bayliss, R. et al., 2004; Bertolin, G. et al., 2016; Cowley, D. O. et al., 2009; Kufer, T. A. et al., 2003; Magnaghi-Jaulin, L. et al., 2019; Ohashi, S. et al., 2006; Zorba, A. et al., 2014).

The opposing activities of the kinase AURKA and phosphatase PP1 determine protein phosphorylation (Meadows, J. C., 2013; Ohashi, S. et al., 2006). PP1 dephosphorylates AURKA, resulting in its deactivation (Meadows, 2013; Ohashi et al., 2006). This establishes a negative feedback loop between AURKA and PP1. Previous reports show that AURKA and PP1 establish this negative feedback loop during

cell division and also suggest that they interact during centrosome maturation (Carmena, M., & Earnshaw, W. C., 2003; Joukov, V. et al., 2014; Meadows, 2013; Ohashi et al., 2006; Nasa, I., 2017). As such, AURKA and PP1 oppose one another for precise control of phosphorylation levels at the mitotic spindle and as well as the centrosome.

F3. PP1, a Central Phosphatase at both Centrosome and Midbody

PP1 is a well characterized phosphatase, with roles that span a broad spectrum of cellular functions (Bollen, P. W., 2010; Gao, K. et al., 2018; Nasa, I., 2017). PP1 does not have inherent specificity and can dephosphorylate any substrates that interact with its catalytic site. More than 250 PP1 regulatory subunits determine PP1 specificity by targeting PP1 to structures within the cell, including the centrosome and midbody (Bollen, P. W., 2010; Gao, K. et al., 2018). Two examples of PP1 regulatory subunits relevant to this work include PP1's interaction with Cep192 during G_2 phase and Cep55 during cytokinesis (Gao, K. et al., 2018; Nasa, I., 2017). PP1's interaction with Cep192 recruits PP1 to the centrosome and Cep55 recruits PP1 to the midbody (Gao, K. et al., 2018; Nasa I., 2017). It is proposed that recruitment of PP1 to the centrosome brings PP1 close to critical kinases and substrates including pericentrin, AURKA, and Plk1, resulting in their dephosphorylation. One focus of the dissertation is PP1's role in antagonizing AURKA activity during centrosome maturation and cell division. PP1 is a component of the broad midbody interactome and dephosphorylates Cep55 to positively regulate Cep55 midbody recruitment. PP1's role in Cep55 midbody recruitment is necessary for maintaining abscission timing during cytokinesis (Bhowmick, R. et al., 2019; Capalbo, L., et al., 2019; Gao, K. et al., 2018).

F4. PPP1R2 is a Bifunctional Coordinator of AURKA and PP1

PPP1R2, a well-established PP1 regulatory subunit, has been implicated in regulating several cell processes including centrosome separation, cell division, and midbody function (Korrodi-Gregório, L. et al., 2013; Li, M., Satinover, D. L., & Brautigan, D. L, 2007). PPP1R2 specifically regulates PP1 in several

processes including sperm maturation, centrosome separation, and cytokinesis (Goswami, S., Korrodi-Gregório, L., Sinha, N., Bhutada, S., Bhattacharjee, R., Kline, D., Vijayaraghavan, S., 2018; Bollen, M., Peti, W., Ragusa, M. J., & Beullens, M., 2010; Eto, M., Elliott, E., Prickett, T. D., & Brautigan, D. L., 2002; Wang, W., Stukenberg, P. T., & Brautigan, D. L., 2008). PPP1R2 belongs to a large family of PP1 regulatory subunits, including PPP1R42 (phosphoprotein protein phosphatase regulatory subunit 42), which regulates cilia formation from the centrosome (DeVaul, N., Wang, R., and Sperry, A. O., 2013). PPP1R2 is a bifunctional protein that activates AURKA in experiments using recombinant proteins and can regulate PP1 and AURKA simultaneously (Satinover D. L., 2004).

F5. PLK1 in Centrosome Maturation

Polo-like Kinase 1 (Plk1) belongs to a large family of related kinases and is responsible for regulating cell cycle checkpoints at S/G_2 as well as G_2/M (Archambault, V. et al, 2015; Colicino, E. G., & Hehnly, H. 2018; Combes, G. et al., 2017; Kumar, S. et al., 2017). Plk1 phosphorylates substrates at these checkpoints, which leads to irreversible transitions between cell cycle phases. It is one of the critical kinases that coordinates the cell cycle with the centrosome cycle due to its overlapping roles in regulating cell signaling pathways as well as centrosome function (Joukov, V. et al., 2014). During G_2 phase, Plk1 directly phosphorylates both Nedd1 and Cep192 to recruit γ-TURC to the centrosome (Joukov, V. et al., 2014). In addition, Plk1 plays major roles in midbody protein recruitment during cytokinesis (Adriaans, I. E. et al., 2019; Bastos, R. N., & Barr, F. A., 2010; Fabbro, M., et al., 2005; Petronczki, M., Lénárt, P., & Peters, J. M., 2008; Takaoka, M. et al., 2014).

G. Dissertation Hypothesis and Aims

The goal of this dissertation research is to define the intricate signaling pathways responsible for centrosome behavior and to determine how dysregulation of these pathways leads to centrosome

dysfunction. My central hypothesis is that *PPP1R2 is a key regulator of the centrosome cycle through its interactions with AURKA and PP1*. The central hypothesis will be tested in the following aims:

Aim #1: Evaluate the effects of PPP1R2, PP1, and AURKA overexpression on the localization and function of proteins associated with the centrosome. I hypothesized that overexpression of PPP1R2, PP1, and AURKA would perturb centrosome function and alter localization of γ-tubulin as well as pericentrin. Results from experiments testing this hypothesis will reveal the effects of PPP1R2, PP1, and AURK overexpression on the structure of the PCM, microtubule nucleation, γ-tubulin localization, and levels of γ-tubulin in the cytoplasm. Results from experiments testing this hypothesis will also reveal whether PPP1R2 co-overexpression with either AURK or PP1 alters localization of γ-tubulin and pericentrin.

Aim #2: Assess the effects of PPP1R2 overexpression on enzyme activity and phosphorylation at the centrosome. I hypothesize that PPP1R2 overexpression will decrease PP1 activity and increase AURKA activity, based on previous reports of PPP1R2's regulation of PP1 and AURKA activities (Li, M., Satinover, D. L., & Brautigan, D. L., 2007; Satinover, D. L., Leach, C. A., Stukenberg, P. T., & Brautigan, D. L., 2004). Results from experiments testing this hypothesis will reveal whether PPP1R2 overexpression changes AURKA or PP1 phosphorylation and activity. I also hypothesized that PPP1R2 overexpression decreases protein phosphorylation at the centrosome. Results from experiments testing this hypothesis will assess the effect of PPP1R2 overexpression on Plk1, AURKA, or PP1 phosphorylation at the centrosome.

Aim #3: Determine PPP1R2's role in midbody regulation. I hypothesized that PPP1R2 regulates PP1 midbody recruitment to maintain proper midbody architecture. Further, I hypothesized that overexpression of PPP1R2 and PPP1R2 dominant negative mutants will alter PP1 midbody localization. Results from experiments will evaluate PPP1R2 overexpression's effect on midbody length and frequency of abnormal midbody morphology.

Summary

Protein phosphorylation is a critical mechanism that regulates protein recruitment to both the centrosome and midbody. Phosphorylation governs centrosome assembly and function during centrosome maturation and mitotic spindle assembly through regulation of protein recruitment. Kinase and phosphatase enzymes such as PP1, AURKA, and Plk1 play critical roles in both centrosome and cell cycle regulation. Enzymes like PP1, AURKA and Plk1 that share targets at both the centrosome and cell cycle complexes link centrosome cycle events to the cell cycle through protein phosphorylation. Phosphorylation-dependent protein recruitment also maintains midbody architecture and function during cytokinesis. Protein phosphorylation is maintained by balanced activity of phosphatases and kinases which are in turn regulated by both phosphorylation and interaction with regulatory subunits.

The focus of this dissertation is to define PPP1R2 regulation of centrosome structure and function as well as cell division. Results from this project demonstrated that PPP1R2 coordinates PP1 and AURKA activity to regulate events at both the centrosome and midbody. Overall, the results from this dissertation research establish a model that clearly outlines how regulatory subunits modulate phosphorylation levels through coordination of critical kinase and phosphatase activities within the context of cell division.

CHAPTER II: THE PP1 REGULATOR PPPP1R2 COORDINATELY REGULATES AURKA AND PP1 TO CONTROL CENTROSOME PHOSPHORYATION AND MAINTAIN CENTRAL SPINDAL ARCHITECTURE

This chapter is modified and reprinted from Bresch, A. M., et al (2020). The PP1 regulator PPP1R2 coordinately regulates AURKA and PP1 to control centrosome phosphorylation and maintain central spindle architecture. *BMC Molecular and Cell Biology*, 2020, 21(1):84, with open permissions from Springer Nature.

A. Summary

Background. Maintenance of centrosome number in cells is essential for accurate distribution of chromosomes at mitosis and is dependent on both proper centrosome duplication during interphase and their accurate distribution to daughter cells at cytokinesis. Two essential regulators of cell cycle progression are protein phosphatase 1 (PP1) and Aurora A kinase (AURKA), and their activities are each regulated by the PP1 regulatory subunit, protein phosphatase 1 regulatory subunit 2 (PPP1R2). I observed an increase in centrosome number after overexpression of these proteins in cells. Each of these proteins is found on the midbody in telophase and overexpression of PPP1R2 and its mutants increased cell ploidy and disrupted cytokinesis. This suggests that the increase in centrosome number I observed in PPP1R2 overexpressing cells was a consequence of errors in cell division. Furthermore, overexpression of PPP1R2 and its mutants increased midbody length and disrupted midbody architecture. Additionally, I show that overexpression of PPP1R2 alters activity of AURKA and PP1 and their phosphorylation state at the centrosome.

Results. Overexpression of PPP1R2 caused an increase in the frequency of supernumerary centrosomes in cells corresponding to aberrant cytokinesis reflected by increased nuclear content and cellular ploidy. Furthermore, AURKA, PP1, phospho PPP1R2, and PPP1R2 were all localized to the midbody at telophase, and PP1 localization there was dependent on binding of PPP1R2 with PP1 and AURKA as well as its phosphorylation state. Additionally, overexpression of both PPP1R2 and its C-terminal AURKA binding site altered enzymatic activity of AURKA and PP1 at the centrosome and disrupted central spindle structure.

Conclusions: Results from our study reveal the involvement of PPP1R2 in coordinating PP1 and AURKA activity during cytokinesis. Overexpression of PPP1R2 or its mutants disrupted the midbody at cytokinesis causing accumulation of centrosomes in cells. PPP1R2 recruited PP1 to the midbody and interference with its targeting resulted in elongated and severely disrupted central spindles supporting an important role for PPP1R2 in cytokinesis.

B. Introduction

The centrosome is a nonmembrane-bounded cytoplasmic organelle that nucleates radial microtubule arrays in both interphase and mitosis. Centrosome function establishes cell polarity, assembles the mitotic spindle to faithfully segregate chromosomes at mitosis, and aligns the midbody during cell division (Fujita, H., 2016; Khodjakov, A., & Rieder, C. L., 2001). Centrioles duplicate once per cell cycle at S-phase, separate, undergo maturation during G2-phase characterized by the recruitment of proteins to the pericentriolar matrix (PCM), and then nucleate microtubules to form the mitotic spindle. It is essential that the cell maintain a normal number of centrosomes, a process dependent on proper duplication of centrosomes along with their accurate distribution at cell division. Aberrant centrosome number leads to multipolar spindles, improper

cell division, aneuploidy, and is strongly correlated with cancer (Levine, M. S. et al., 2017; Rivera-Rivera, Y., & Saavedra, H. I., 2016).

These events are dependent, in part, on the activity of Aurora A kinase (AURKA) and protein phosphatase 1 (PP1) (Moura, M., & Conde, C., 2019). PP1 regulates a myriad of cellular processes some tied to centrosome biology including mitosis, cytokinesis, and the cell cycle (Bhowmick, R., 2019; Ceulemans, H., 2004; Gao, K. et al., 2018; Moura, M., & Conde, C. 2019). PP1's roles are specified by binding of its catalytic subunit to as many as 200 different regulatory subunits (Bollen, Peti, Ragusa, & Beullens, 2010; Ceulemans H, 2004; Moura & Conde, 2019). Interactions with specific regulatory subunits and centrosome scaffolding proteins recruit PP1 to the centrosome and midbody (Bhowmick, R., 2019; Gao, K. et al., 2018; Nasa, I., 2017). Centrosome number depends on regulation of both centrosome duplication and cytokinesis; both events rely on the balanced activities of protein phosphatases and kinases (Fujita, H. Y., 2016; Helps, N. R., Luo, X., Barker, H. M., & Cohen, P. T., 2000; Meraldi, P., & Nigg, E. A., 2001).

One PP1 regulatory subunit, PPP1R2, was identified as a heat-stable inhibitor of PP1 (Connor, J. H. et al., 2000; Fujita, H. Y., 2016; Helps, N. R. et al., 2000; Holmes, C. F., Campbell, D. G., Aitken, A., & Cohen, P., 1986; Meraldi, P., & Nigg, E. A., 2001). PPP1R2 activity peaks during mitosis, where it regulates centrosome separation, chromosome segregation, and cytokinesis (Brautigan, D. L. et al., 1990; Carmena, M., & Earnshaw, W. C., 2003; Eto, M., Elliott, E., Prickett, T. D., & Brautigan, D. L., 2002; Satinover, D. L., Brautigan, D. L., & Stukenberg, P. T., 2006). The PP1-PPP1R2 complex induces centrosome separation prior to mitosis through association with 'Never in Mitosis Kinase 2' (NEK2) (Helps, N. R. et al., 2000; Mi, J., Guo, C., Brautigan, D. L., & Larner, J. M., 2007). PPP1R2 is a bifunctional protein – in addition to

inhibiting PP1, PPP1R2 also regulates the G2/M transition by binding and activating AURKA (Satinover, D. L., 2006; Satinover, D. L. et al., 2006). AURKA is a highly conserved serine-threonine kinase that regulates centrosome maturation, spindle formation, and cytokinesis (Carmena, M., & Earnshaw, W. C., 2003). Loss of AURKA function results in monopolar spindles and bipolar spindles with multiple centrosomes at one pole, indicating that centrosomes fail to segregate in the absence of AURKA (Giet, R. et al., 2002; Glover, D. M. et al., 1995). AURKA overexpression causes centrosome multiplication through failure of cytokinesis in cultured mammalian cells and occurs along with centrosome amplification in cancer (Karthigeyan, P. S., 2011; Meraldi, P., Honda, R., & Nigg, E. A., 2002; Nikonova, A. S., Astsaturov, I., Serebriiskii, I. G., Dunbrack,, R. L., & Golemis, E. A., 2013; Zhou, H. et al., 1998). PP1 and its subunits counteract activities of the mitotic kinases AURKA and AURKB to maintain proper spindle formation and chromosome segregation during mitosis (Eto, M. et al., 2002; Katayama, Z. H., 2001; Lioutas, A., & Vernos, I., 2013; Mi, J. et al., 2007; Wang, W., Stukenberg, P. T., & Brautigan, D. L., 2008).

While it is known that PPP1R2 interacts with both AURKA and PP1, it is unclear how these activities might affect the number of centrosomes in the cell. The aims of this study were: 1) to determine how interaction between PPP1R2, PP1, and AURKA affects centrosome number, 2) to investigate the role of PPP1R2 as a known regulator of both PP1 and AURKA in maintenance of centrosome number, and 3) to investigate a role for PPP1R2 in cytokinesis. Our results indicate PP1 and PPP1R2 oppose AURKA activity to counter AURKA's induction of multiple centrosomes in cells. A phosphomimetic mutant of PPP1R2 induced supernumerary centrosomes, suggesting that PPP1R2 phosphorylation may be required to interact with and regulate PP1 and

AURKA to prevent an increase in centrosome number. I show that both PP1 and AURKA binding domains on PPP1R2 are each important to maintain the normal number of centrosomes, supporting the proposal that interaction of PPP1R2 with AURKA and PP1 is important to maintain centrosome number. In addition, overexpression of PPP1R2 increased nuclear size indicating that PPP1R2 may regulate cytokinesis. Consistent with previous reports demonstrating a role for PPP1R2 in cytokinesis (Wang, W. et al., 2008), I localized PPP1R2 to the midbody at telophase, along with AURKA and PP1 that have also been previously shown to regulate cytokinesis (Bhowmick, T. R., 2019; Gao, K. et al., 2018; Mangal, S. et al., 2018; Meraldi, P. et al., 2002). Overexpression of PPP1R2 and PPP1R2 phosphomimetic enhanced targeting of PP1 to the midbody and increased centrosome number while overexpression of PPP1R2 truncation mutants caused elongated and distorted central spindles. Together, our findings demonstrate that both PPP1R2 phosphorylation and its interaction with AURKA and PP1 are necessary to direct PP1 to the midbody in an ideal location to regulate events prior to abscission.

C. Experimental Procedures

C1. Cell Culture and DNA Transfection

Human pigmented retinal epithelial cells (ARPE-19; American Type Tissue Collection) were grown in DMEM-F12 media supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. The PPP1R2 overexpressing plasmid was constructed by inserting the *Ppp1R2* coding sequence (a gift of Dr. Srinivasan Vijayaraghavan, Kent State University) in frame with the FLAG tag of the mammalian expression vector CMVFLAG 3X-14 (Sigma Aldrich). The PP1 plasmid was a gift of Dr. James McDonald, Western University, Cancer Research Center. The AURKA plasmid was obtained from Dr. Eric Nigg, University of Basil. PPP1R2 phospho-mutants

were generated using the QuikChange® site-directed mutagenesis kit (Stratagene) and the PPP1R2 deletion mutants were obtained from Dr. David Brautigan, University of Virginia. ARPE-19 cells plated on glass coverslips were grown to approximately 70% confluence then grown for 24 hours prior to transfection. Cells were transfected using Lipofectamine-2000 (Invitrogen) according to manufacturer's recommendations.

C2. Immunofluorescence and Measurement of Protein Localization.

Transfected cells were fixed and permeabilized with methanol, then nonspecific binding was blocked by incubation in 3% BSA in TBST (20 mM Tris, pH 7.5, 150 mM NaCl, 2 mM EGTA, 0.1% Triton X-100) for 30 minutes. The cells were incubated overnight at 4°C with primary antibody, then with secondary antibodies conjugated to Alexa Fluor-488 and 594 (1:200, Life Technologies). α-tubulin was detected with a goat polyclonal antibody (1:200, 74010 clone TUBA4A, Life Sciences), PPP1R2 with a rabbit polyclonal antibody (1:100, 851753, MyBioSource), pPPP1R2 with a rabbit polyclonal antibody (1:100, 44-1160G, Thermo Fisher Scientific), AURKA with a rabbit polyclonal antibody (1:100, PA5-34700,Thermo Fisher Scientific), pAURKA rabbit polyclonal antibody (1:100, 44-1210G, Thermo Fisher Scientific), PP1 with a rabbit polyclonal antibody (1:100, L5-454752, Lifespan Biosciences), pPP1 rabbit polyclonal antibody (1:100, PA5-17819, Thermo Fisher Scientific), FLAG with a rabbit polyclonal antibody (1:500, PA1984B; Thermo Fisher Scientific), c-myc with a rabbit polyclonal antibody (1:500, NB600-335, Novus Biologicals), and γ-tubulin with a mouse monoclonal antibody (1:50, PA5-34815, Thermo Fisher Scientific). DNA was labeled with Vectashield mounting media containing 4′, 6-diamidino-2-phenylindole (DAPI) dye (Vector Laboratories).

The intracellular localization of proteins was visualized using a Nikon E600 fluorescence microscope, Pan Fluor 100X objective (N.A. 0.5-1.3) or Pan Fluor 40X objective (N.A. 0.75), fit with appropriate filters. Images were captured with an Orca II CCD camera (Hamamatsu) and Metamorph image analysis and acquisition software (Universal Imaging Corporation). Images were exported to ImageJ (NIH) and only linear adjustments to brightness and/or contrast were performed.

Midbody protein localization was quantified using Metamorph software. Parameters were set by using 10 x 10-pixel sized squares with each having an area of 0.87 μm^2. 10 squares were aligned across the midbody at intervals of 1 μm starting from the midbody as a central orienting landmark.

C3. DNA Content Measurement

Cells were transfected with FLAG, PPP1R2, or AURKA plasmids, fixed with 70% ethanol, and stained with propidium iodide. DNA content was assessed using a cell flow cytometer (Becton Dickinson FACScan Cytometer) and CellQuest (BD biosciences) software. Cell counts were capped at 3000 for each run and parameters set to exclude all doublet cells and cells with expected DNA content. Remaining polyploid cells were counted and divided by total cell count to calculate percentage of cells with abnormal ploidy levels.

C4. Cell Lysate Preparation and ELISA

Transfected ARPE cells were collected using a cell lifter and solubilized in cell lysis buffer (50 mM Tris, 1mM EGTA, 1% NP40). Cell lysate was treated with 1:100 Halt phosphatase inhibitor cocktail (Sigma-Aldrich), 1:10 protease inhibitor cocktail (Calbiochem) and lysed using a freeze-thaw method using liquid nitrogen. Following freeze-thaw, benzonase nuclease (Novagen) digested nuclear material in the lysate. Protein concentration of the lysate was measured using a Pierce BCA protein assay kit (Thermofisher).

96-well plates (Falcon) were coated with cell lysate at a protein concentration of 20ug/ml; 1 ug of protein per well. Plates were blocked with 3% BSA solution diluted in phosphate buffer buffered saline (PBS, 13.7 mM NaCl, 2.7 mM KCl, 1.4 mM KH_2PO_4, 4.3 mM Na_2HPO_4). Plates were then incubated at 4ºC overnight and then washed with 1x PBS. Cells were then incubated with primary antibodies targeted to FLAG (1:500, F1804, Sigma Aldrich), AURKA (1:500, IHC-00062, Thermofisher), PP1 (1:500, A-300-904A-M Thermofisher), pPP1 (1:500, 25815, Cell Signaling) at 4ºC overnight. Cells were washed and incubated with donkey anti-rat (1:2,000, Jackson Immuno Research Lab) and donkey anti-mouse (1:2,000, Jackson Immuno Research Lab) HRP conjugated secondary antibodies for one hour at room temperature. After a final wash, bound antibody was detected with o-phenylenediamine dihydrochloride (OPD) 1g/L in buffer (0.05M citric acid, 0.05M sodium phosphate, 1% hydrogen peroxide, pH 5) for thirty minutes at 37ºC. Fluorescence was then measured using a plate reader fitted with a 450 nm filter.

C5. AURKA Immunoprecipitation and Activity Assay, Phosphatase Activity Assay

Protein complexes were collected by immunoprecipitation using Sepharose bead-antibody capture. Briefly, affinity purified antibody to AURKA (1:500, IHC-00062, Thermofisher) was incubated with precleared cell lysate (>1 mg protein) followed by anti-rabbit IgG beads. Immunoprecipated proteins were detected by ELISA with anti-AURKA antibody (1:500, IHC-00062, Thermofisher), and anti-HRP (1:2000, 131366 Abcam). Negative control for coimmunoprecipitation was a sample without antibody.

AURKA activity was detected using the Universal Kinase Assay Kit on samples obtained from AURKA immunoprecipitation (abcam, ab138879). Samples were prepped and processed through the kinase assay kit as per manufacturer's instructions. Samples were measured by plate reader at 540/590 nm excitation/emission.

PP1 activity was detected using a RediPlate^{TM} 96 EnzChek$^{®}$ Serine/Threonine Phosphatase Assay Kit as per manufacturer's instruction (Thermofisher). Cell lysate was prepared following transfection and phosphatase activity in equal amounts of protein measured by plate reader at 355/460 nm excitation/emission.

C6. Statistical Analyses

The data for centrosome quantitation was expressed as mean ± SEM. The differences between groups were analyzed using a One-way ANOVA and unpaired Student's t test with JMP Version 13.1. Differences at $p \leq 0.05$ were considered statistically significant. I used the software Q*Power to perform calculate sample sizes appropriate for 80% power and an alpha value below 0.2.

D. Results

D1. PPP1R2, AURKA, and PP1 Interact to Affect Centrosome Number.

PPP1R2 regulates PP1 and AURKA, both of which are essential to maintain proper centrosome number (Bhowmick R, 2019; Eto et al., 2002; Gao et al., 2018; Liu & Ruderman, 2006; Lukasiewicz & Lingle, 2009; Mangal et al., 2018; Meraldi et al., 2002; Mi et al., 2007; Nikonova AS, 2013; Peel N, 2017; Zhou et al.. To better define if PPP1R2 interacts with AURKA and PP1 to maintain centrosome number, I overexpressed epitope-tagged proteins in ~85% of ARPE-19 cells (Figure 2.1A-B). All plasmids used in this study were expressed to similar levels in ARPE-19 cells (Figure 2.2). Overexpression of PPP1R2 as well as AURKA and PP1 induced supernumerary centrosomes, with some cells having as many as 6 centrosomes, visualized as γ-tubulin puncta (Figure 2.1D-F, inset) compared to 1-2 found in empty vector controls (Figure 2.1C, inset). The frequency of supernumerary centrosomes in cells overexpressing PPP1R2 increased 8-fold compared to cells transfected with empty vector ($p \leq 0.0001$) (Figure 2.1J). Co-overexpression of AURKA and PPP1R2 restored supernumerary centrosome frequencies to control levels (Figure 2.1G inset, J). In contrast, co-overexpression of PP1 and PPP1R2 only partially restored supernumerary centrosome frequency (Figure 2.1I inset, J).

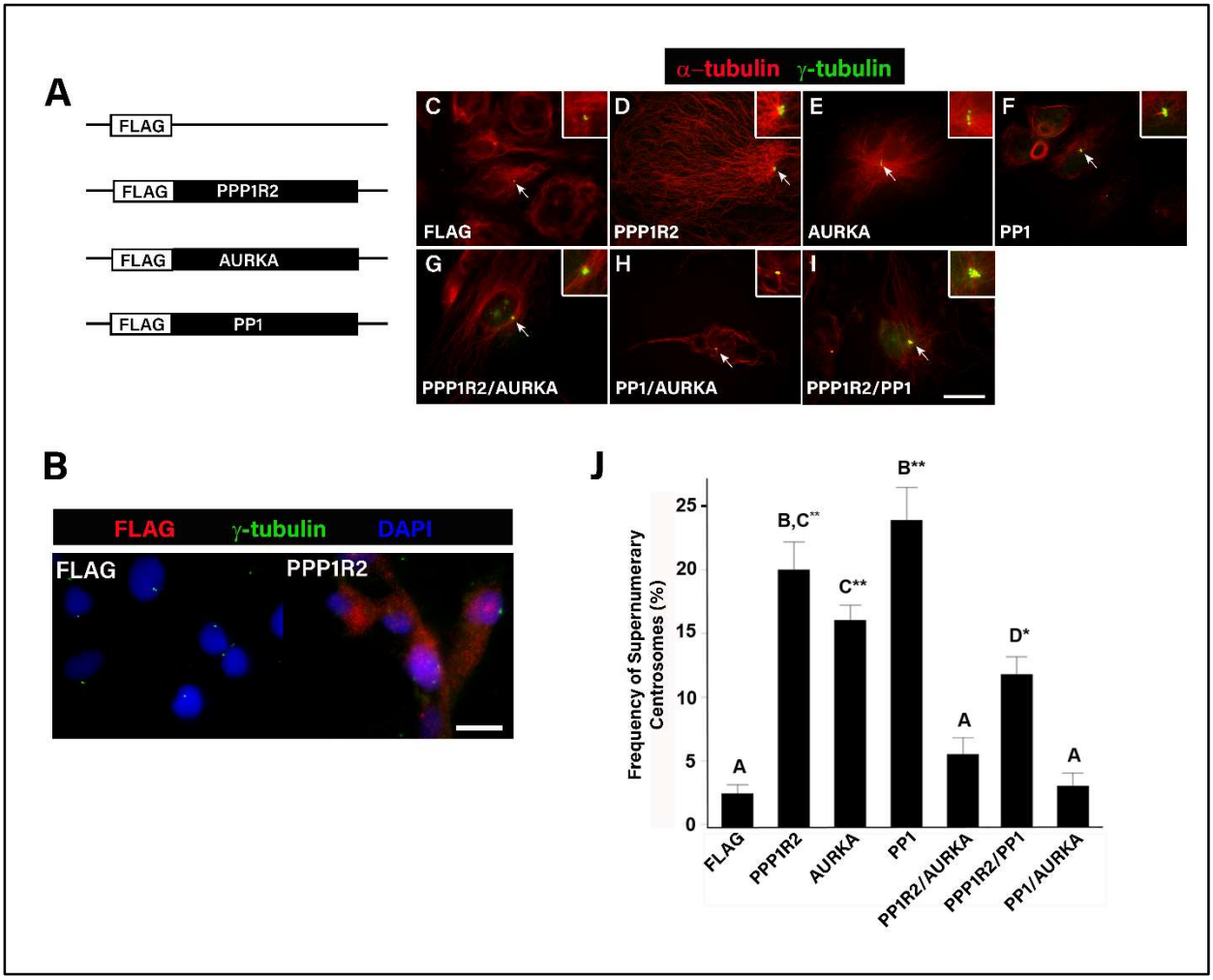
A
FLAG
FLAG PPP1R2
FLAG AURKA
FLAG PP1
α–tubulin γ-tubulin
C
FLAG
D
PPP1R2
E
AURKA
F
PP1
G
PPP1R2/AURKA
H
PP1/AURKA
I
PPP1R2/PP1
B
FLAG γ-tubulin DAPI
FLAG
PPP1R2
J
Frequency of Supernumerary Centrosomes (%)
25
20
15
10
5
0
A
B,C**
C**
B**
A
D*
A
FLAG
PPP1R2
AURKA
PP1
PP1R2/AURKA
PPP1R2/PP1
PP1/AURKA

Figure 2.1. PPP1R2 affects centrosome number through interaction with AURKA and PP1. (A-B) Schematic of constructs used for transfection and representative transfection. (C-I) ARPE-19 cells were transfected either singly or in combination with plasmids expressing PPP1R2, AURKA, PP1 and empty vector as control (FLAG). Transfected cells were stained for γ-tubulin (green) and α-tubulin (red) and centrosomes were counted in a minimum of 100 cells for each treatment group in three replicates. Insets magnify cellular regions containing centrosomes. Size bar equals 10 μm. (J) Graphical representation of the frequency of supernumerary centrosomes in cells transfected with each of the indicated plasmids individually or in combination. Statistically significant differences ($p<0.05$) between groups are indicated by differing letter notations above the bars and error bars represent standard error of the mean. Statistically significant differences are indicated with asterisks (*=$p\leq0.01$, **=$p\leq0.001$) compared to control.

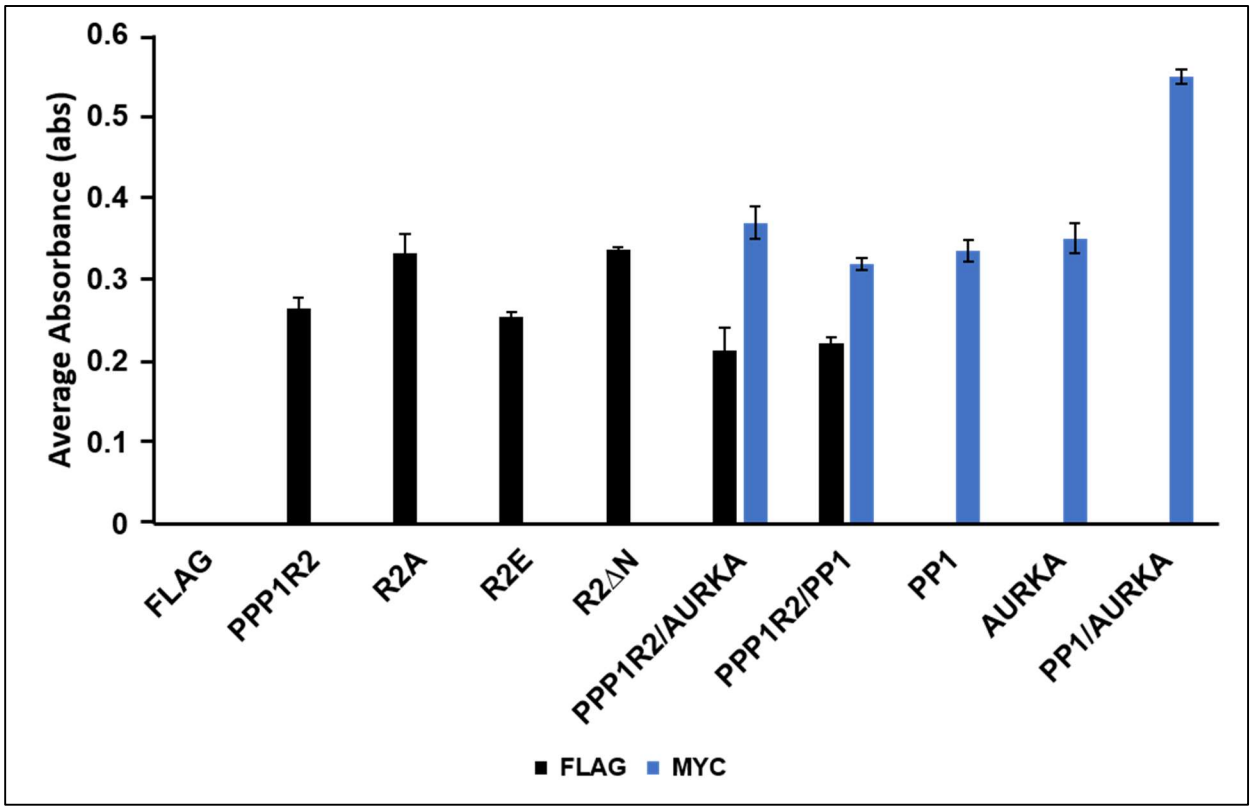
Average Absorbance (abs)
0.6
0.5
0.4
0.3
0.2
0.1
0
FLAG
PPP1R2
R2A
R2E
R2ΔN
PPP1R2/AURKA
PPP1R2/PP1
PP1
AURKA
PP1/AURKA
FLAG
MYC

Figure 2.2. Expression of ectopically expressed tagged proteins. Fusion proteins tagged with either FLAG (black) or myc (blue) were detected in cells by ELISA 24 hours after transfection.

Overexpression of PP1 and AURKA together in cells did not increase centrosome number compared to controls, in contrast to the effect of AURKA and PP1 alone (Figure 2.1E-F inset, J). These results reveal the importance of interaction between PP1 and AURKA as well as between PPP1R2 and both AURKA and PP1 to maintain the correct number of centrosomes in cells.

D2. PPP1R2 Interaction with both PP1 and AURKA Affects Centrosome Number and is Phosphorylation Dependent.

Glycogen synthase kinase-3β is known to inactivate PPP1R2 by phosphorylating threonine 72, thereby activating PP1 (Cohen, 1989; Holmes CF, 1986; Sakashita et al., 2003). In order to test the relevance of this modification to PPP1R2 function, I created a homologous mutation in human PPP1R2, at Thr73 from threonine to either alanine (R2A, which cannot be phosphorylated) or glutamic acid (R2E, a phosphomimetic) as shown in Figure 2.3A (left). These plasmids were transfected into ARPE-19 cells (Figure 2.3C-D) to test whether induction of supernumerary centrosomes by PPP1R2 was dependent upon its phosphorylation state. Compared to control cells (Figure 2.3B, inset), overexpression of the PPP1R2 R2A (R2A) mutant did not increase centrosome numbers (Figure 2.3C inset, G). In contrast, overexpression of the PPP1 R2E (R2E) mutant increased the frequency of supernumerary centrosomes ~7-fold ($p \leq 0.0001$; Figure 2.3D inset, G).

PP1 and AURKA bind to separate domains on the PPP1R2 protein; PP1 binds to the N-terminal region (aa 1-118), while AURKA binds to the C-terminal region (aa 119-197) (Figure 2.3A, right) (Connor et al., 2000; Satinover, Leach, Stukenberg, & Brautigan, 2004). Therefore, I tested the effect of deletion of these binding domains on induction of supernumerary centrosomes. This was accomplished by transfecting PPP1R2 truncation mutants lacking either

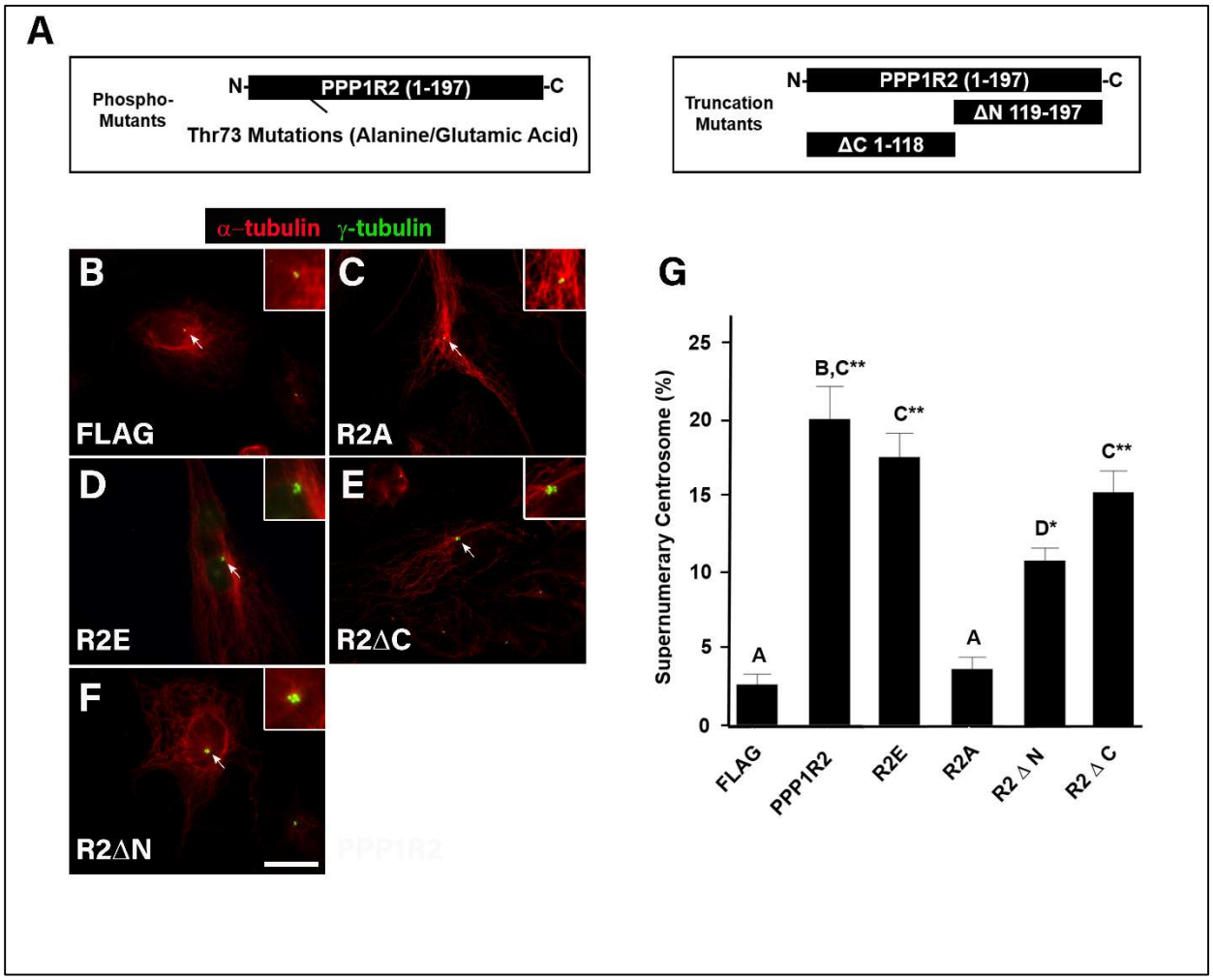
A
Phospho-Mutants
N-
PPP1R2 (1-197)
-C
Thr73 Mutations (Alanine/Glutamic Acid)
Truncation Mutants
N-
PPP1R2 (1-197)
-C
ΔN 119-197
ΔC 1-118
α-tubulin γ-tubulin
B
FLAG
C
R2A
D
R2E
E
R2ΔC
F
R2ΔN
G
Supernumerary Centrosome (%)
25
20
15
10
5
0
A
B,C**
C**
A
D*
C**
FLAG
PPP1R2
R2E
R2A
R2 Δ N
R2 Δ C

Figure 2.3. PPP1R2 interaction with both PP1 and AURKA affects centrosome number and is phosphorylation dependent. (A) Schematics of the PPP1R2 mutants used for transfection. The left schematic shows the position of phosphorylation site mutants involving the Thr73 residue including both the threonine to alanine phosphonull mutation PPP1R2A (R2A) and threonine to glutamic acid phosphomimetic mutation PPP1R2E (R2E). The right schematic indicates position of PPP1R2 truncations which included PPP1R2 plasmids truncated at either the N-terminus PPP1R2ΔN (R2ΔN), deleting the PP1 binding site, or the C-terminus PPP1R2ΔC (R2ΔC), removing the AURKA binding site. These mutants were tested for their effect on centrosome number (C-F). Cells were transfected with the indicated proteins, fixed, and stained with anti-γ-tubulin (green) and anti-α-tubulin (red). Size bar equals 10 μm. Insets show the region containing centrosomes. (G) Quantitation of the percentage of cells with supernumerary centrosomes after transfection with the indicated constructs. Statistically significant differences among groups are indicated by differing letter notations above the bars at $p \leq 0.05$ (*$p \leq 0.01$, **$p \leq 0.001$).

the PP1 (R2ΔN; Figure 2.3A, right) or the AURKA binding site (R2ΔC; Figure 2.3A, right), respectively. All proteins were detectable by ELISA after transfection, except for R2ΔC that lacked an epitope tag (Figure 2.2). Deletion of either the PP1 or the AURKA binding site on PPP1R2 reduced the frequency of supernumerary centrosomes compared to full length PPP1R2 (Figure 2.3E-G), revealing that overexpression of either fragment alone increases centrosome number through their individual interaction with PP1 and AURKA.

D3. PPP1R2 Overexpression Increased Cell Ploidy and Accumulation of Cells with Enlarged Nuclei.

AURKA overexpression was previously shown to induce tetraploidization through cytokinesis failure (Katayama H, 2001; Meraldi & Nigg, 2001; Zhou et al., 1998). Overexpression of PPP1R2 increased the number of cells with increased DNA content, as compared to empty vector controls ($p \leq 0.001$, Figure 2.4A-B). Overexpression of the PPP1R2 R2E phosphomimetic mutant also significantly increased cellular DNA content ($p \leq 0.01$) as compared to cells overexpressing PPP1R2 R2A (R2A) or the empty vector control (Figure 2.4A-B). The number of cells with increased ploidy quadrupled following PPP1R2 overexpression consistent with the observed increase in DNA content (Figure 2.4C). Both R2 C- and N- terminal truncation mutant overexpression resulted in the most significant increase in nuclear content (Figure 2.4B). Our result is similar to that seen when AURKA is overexpressed (Meraldi et al., 2002); therefore, I investigated whether PPP1R2 causes an increase in chromosome number through an effect on cytokinesis.

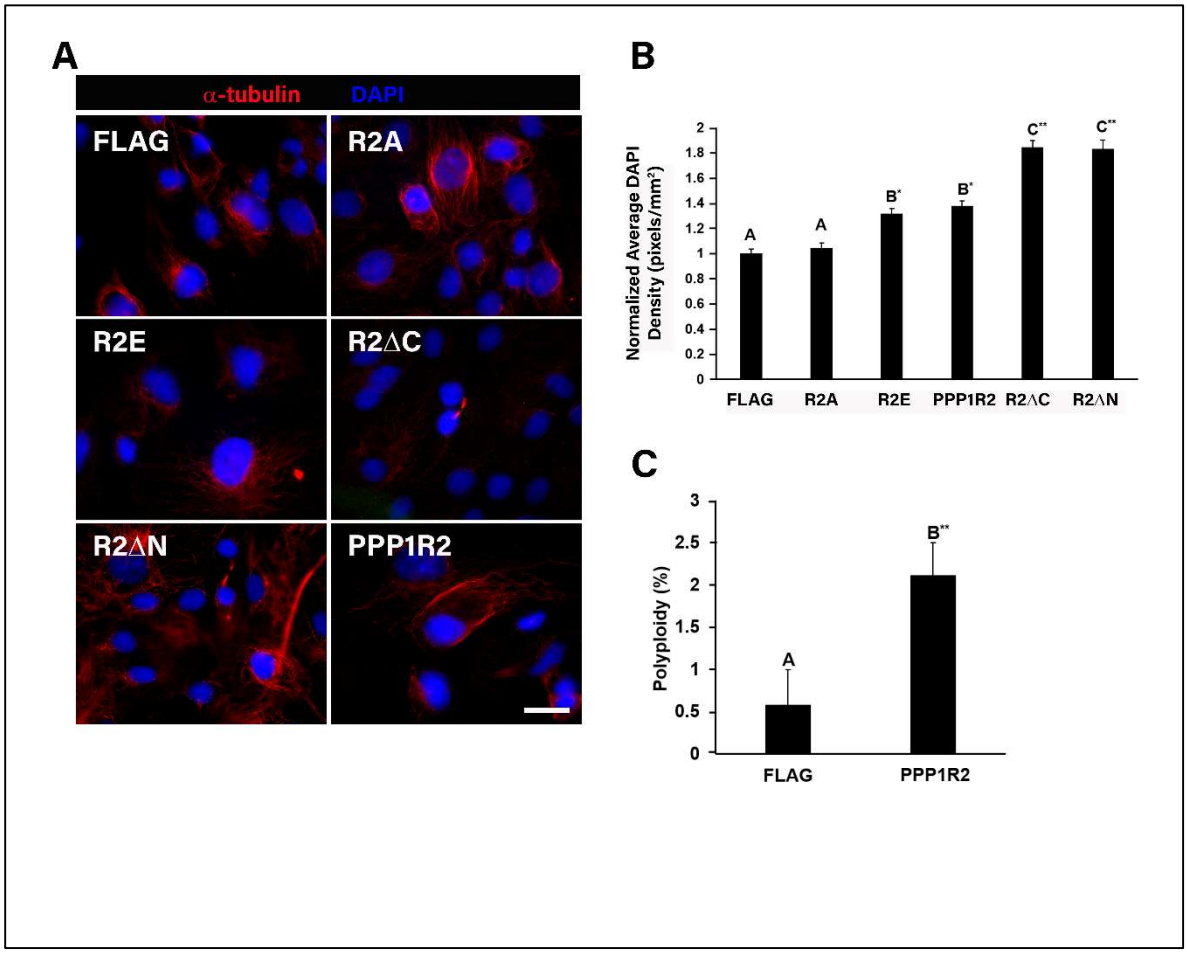
A
α-tubulin
DAPI
FLAG
R2A
R2E
R2ΔC
R2ΔN
PPP1R2
B
Normalized Average DAPI Density (pixels/mm²)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
A
A
B*
B*
C**
C**
FLAG
R2A
R2E
PPP1R2
R2ΔC
R2ΔN
C
Polyploidy (%)
3
2.5
2
1.5
1
0.5
0
A
B**
FLAG
PPP1R2

Figure 2.4. PPP1R2 overexpression increased cell ploidy and accumulation of cells with enlarged nuclei. (A) Cells were transfected with the indicated FLAG-tagged fusion proteins, fixed and stained for α-tubulin to detect microtubules (red), and DAPI to stain the nucleus (blue). Size bars equal 10 μm. (B) Quantitation of DAPI intensity after transfection with the indicated constructs and normalized to the control. (C) Quantitation of cells with increased ploidy after transfection with PPP1R2. Statistically significant differences ($p \leq 0.05$) between experimental groups and empty vector control are indicated by differing letters above the bars and asterisks indicate *$p \leq 0.01$ and **$p \leq 0.001$.

D4. PPP1R2, pPP1R2, PP1, and AURKA are Localized to the Midbody during Cytokinesis.

Given our data and previously established roles of AURKA and PP1 in cytokinesis, I next investigated localization of AURKA, PP1, PPP1R2, and phospho PPP1R2 in dividing ARPE-19 cells. The midbody and central spindle are distinct structures formed during cytokinesis, and I established a scoring system to quantify fluorescent levels of proteins across each structure. AURKA, PP1, pR2 (phospho-PPP1R2), and PPP1R2, were each detected at telophase on the midbody (Figure 2.5A). Fluorescent localization of AURKA, PP1, PPP1R2 and pR2 was quantified along the midbody in equal-sized boxes (Figure 2.5B). PPP1R2, pR2, PP1, and AURKA were all positioned at the midbody (Figure 2.5C), consistent with a shared role in cytokinesis. Interestingly, pR2 localization was more enriched at the midbody than the entire pool of PPP1R2 (Figure 2.5A).

D5. PPP1R2 Targets PP1 to the Midbody.

I next investigated if full length PPP1R2 could increase PP1 location at the midbody and if PPP1R2 mutants could interfere with this targeting. I transfected ARPE-19 cells with full length PPP1R2 or the mutants and measured endogenous PP1 localization at the midbody. PPP1R2 overexpression resulted in a significant ($p<.001$) increase in localization of PP1 at the midbody (Figure 2.6F inset, G-H). Interestingly, PPP1R2 R2A (R2A) (phosphonull) overexpression had no significant effect on PP1 localization when compared to empty vector control (Figure 2.6A-B inset, G-H). In contrast, PPP1R2 R2E (R2E) (phosphomimetic) overexpression significantly ($p<0.01$) enhanced PP1 localization at the midbody (Figure 2.6C inset, G-H) compared to both empty vector control and R2A mutant overexpression (Figure 2.5, 2.6). This difference suggests PPP1R2 phosphorylation directs PP1 recruitment to the midbody. R2ΔN and R2ΔC overexpression

significantly reduced PP1 midbody localization, as compared to empty vector control ($p<0.001$) (Figure 2.6D-E, G-H) Overall, both N-terminal and C-terminal domains were necessary for PP1 recruitment to the midbody at telophase and phosphorylated PPP1R2 is critical to this process.

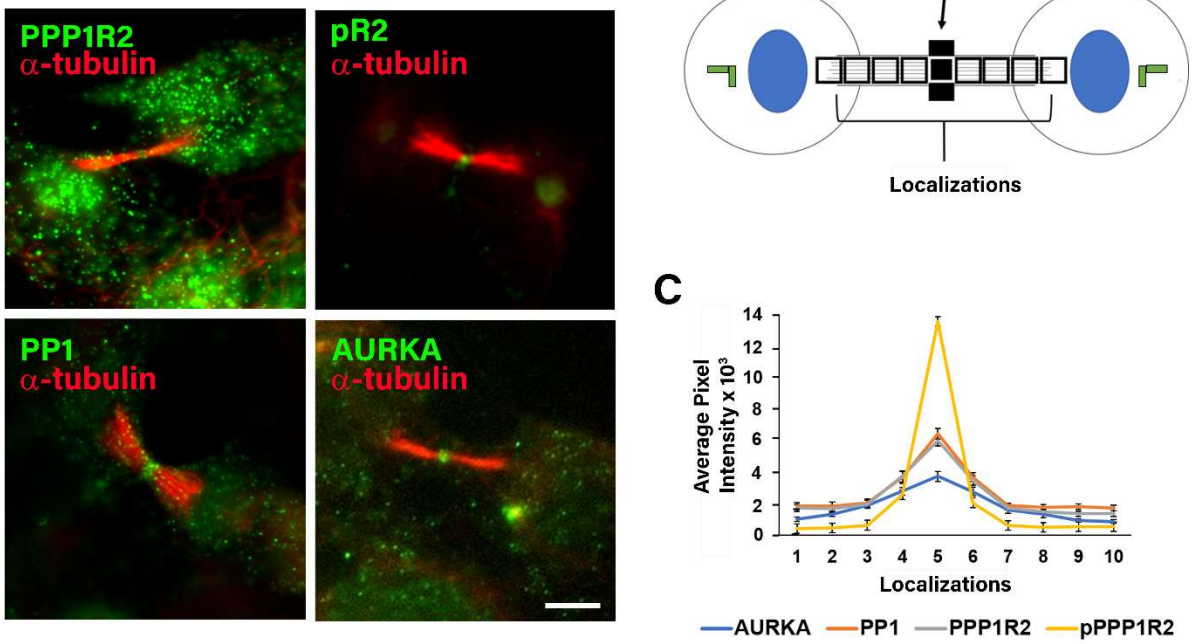
A
PPP1R2
α-tubulin
pR2
α-tubulin
PP1
α-tubulin
AURKA
α-tubulin
B
Midbody Ring
(Localization 5)
Localizations
C
Average Pixel Intensity x 10³
14
12
10
8
6
4
2
0
1 2 3 4 5 6 7 8 9 10
Localizations
AURKA
PP1
PPP1R2
pPPP1R2

Figure 2.5. PP1, PPP1R2, pR2, and AURKA are localized at the midbody. (A) ARPE-19 cells were fixed and stained for the indicated endogenous proteins (green) localized relative to α-tubulin of the central spindle (red). (B) Average intensity from the green channel along the central spindle was measured in 10-0.87 μm^2 square regions spanning the length of the central spindle in 30 dividing cells

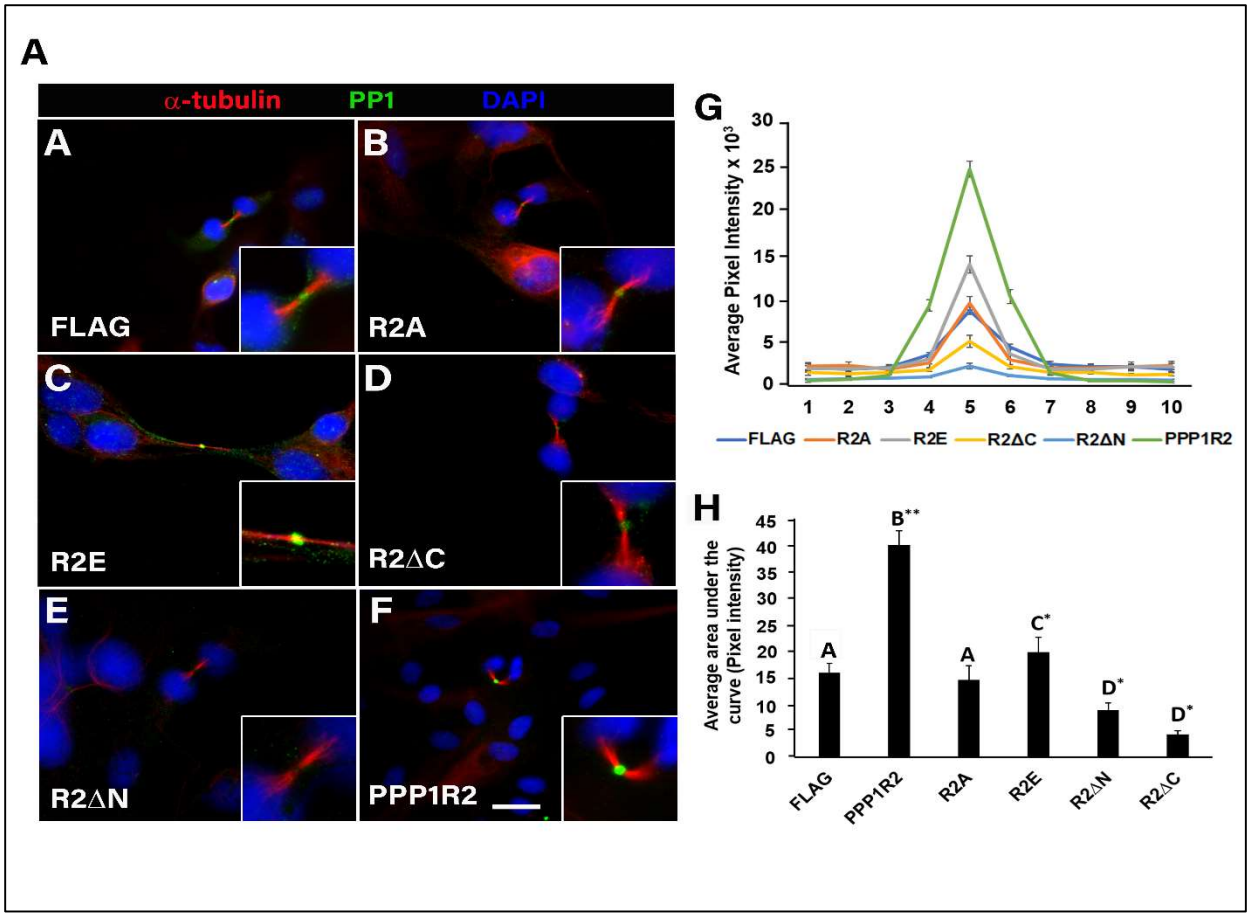
A
α-tubulin
PP1
DAPI
A
FLAG
B
R2A
C
R2E
D
R2ΔC
E
R2ΔN
F
PPP1R2
G
Average Pixel Intensity x 10³
30
25
20
15
10
5
0
1 2 3 4 5 6 7 8 9 10
FLAG R2A R2E R2ΔC R2ΔN PPP1R2
H
Average area under the curve (Pixel intensity)
45
40
35
30
25
20
15
10
5
0
A
B**
A
C*
D*
D*
FLAG
PPP1R2
R2A
R2E
R2ΔN
R2ΔC

Figure 2.6. PPP1R2 targets PP1 to the midbody. (A-F) ARPE-19 cells were transfected, fixed, and labeled for PP1 (green) to determine the localization of endogenous PP1 along the central spindle (red) following overexpression of PPP1R2 mutants: a phosphonull Thr73 mutant PPP1R2A (R2A), a phosphomimetic Thr73 mutant PPP1R2E (R2E), a C-terminal truncation PPP1R2ΔC (R2ΔC), and an

D6. PPP1R2 Mutant Overexpression Altered both Central Spindle Length and Structure.

I investigated if overexpression of PPP1R2 and PPP1R2 mutants affected central spindle length and observed elongated central spindles in cells overexpressing these constructs (Figure 2.7). Cells overexpressing PPP1R2 or its mutants displayed central spindles that were on average >20% longer than controls. This was observed in both phosphomimetic as well as truncated mutant overexpression (Figure 2.7A-B). Truncation mutants resulted in significantly longer central spindles compared to all other treatment groups (Figure 2.7B, $p<0.001$). Additionally, overexpression of PPP1R2 mutants significantly increased the frequency of abnormal central spindle morphology in transfected cells (Figure 2.8A-B, $p<0.01$). Abnormal central spindle morphology was defined as those with either an irregular tortuous structure or unraveled microtubules; these phenotypes were rarely seen in control treated cells. Although the frequency of disrupted central spindles increased to the same level as full length PPP1R2 for each of the mutants compared to control (Figure 2.8B), the severity of misshapen central spindles was most pronounced in cells overexpressing the truncation mutants (Figure 2.8A, R2ΔC and R2ΔN). The high degree of disruption seen in the truncation mutants correlated with an increase in central spindle length compared to other constructs (Figure 2.7B). I propose that the increase in central spindle length contributes to distortion of this structure in the truncation mutants. Microtubule bundling was profoundly disrupted in the central spindle in the region closest to DNA in cells overexpressing each PPP1R2 mutant. Altogether this data suggests that PPP1R2 has a role in maintaining central spindle architecture.

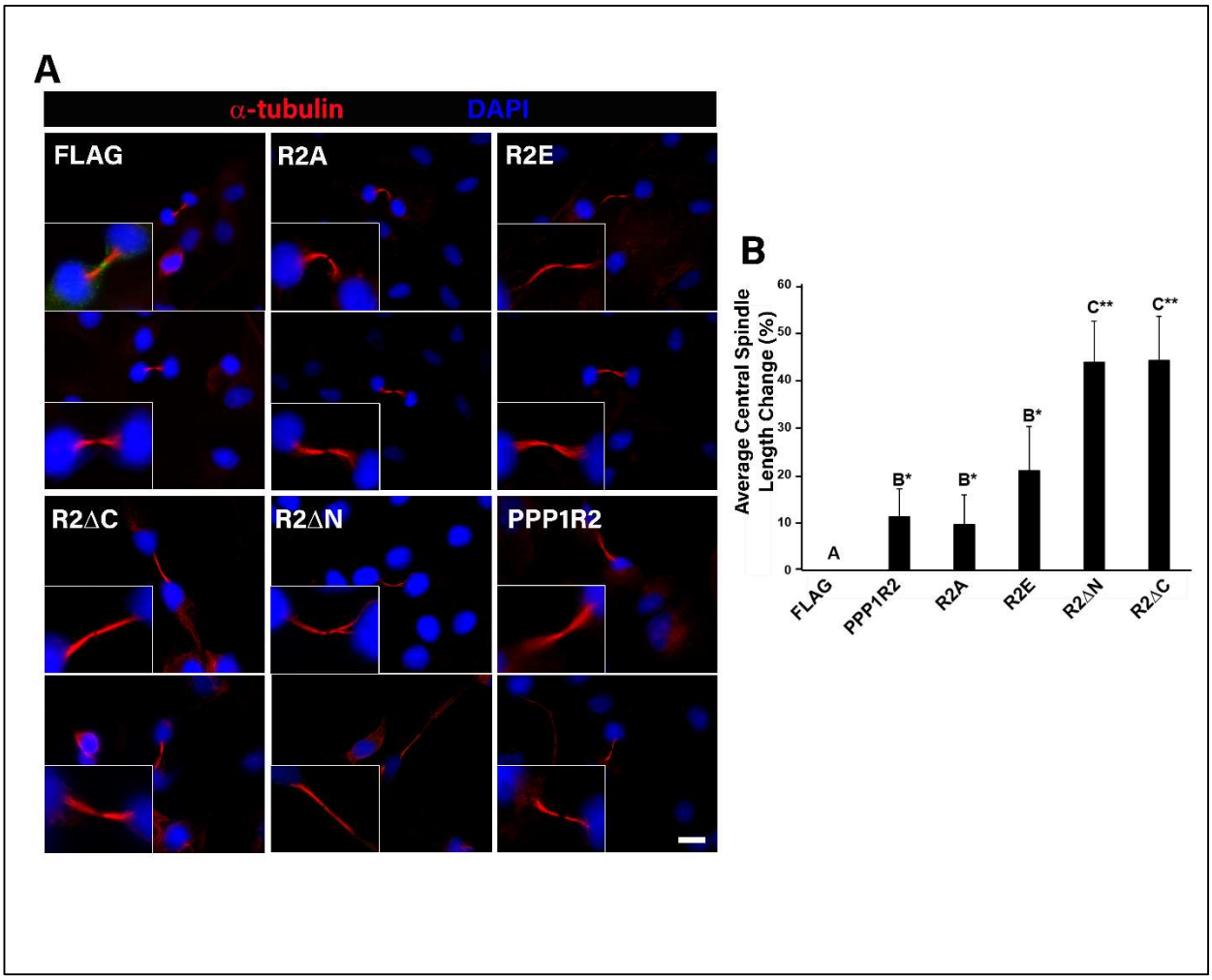
A
α-tubulin
DAPI
FLAG
R2A
R2E
R2ΔC
R2ΔN
PPP1R2
B
Average Central Spindle Length Change (%)
60
50
40
30
20
10
0
A
B*
B*
B*
C**
C**
FLAG
PPP1R2
R2A
R2E
R2ΔN
R2ΔC

Figure 2.7. PPP1R2 mutant overexpression increased midbody length. (A) ARPE-19 cells were transfected with FLAG, PPP1R2, and PPP1R2 mutants (R2A, R2E, R2ΔC, R2ΔN), fixed, and labeled with DAPI (blue) as well as α-tubulin (red) to stain the central spindle. Size bar equals 5μm. (B) Midbody length was quantified using Metamorph software and averages were calculated within three biological replicates (n>30 dividing cells). Statistically significant differences ($p \leq 0.05$) between experimental groups and empty vector control are indicated by differing letters above the bars and asterisks indicate *$p \leq 0.01$ and **$p \leq 0.001$.

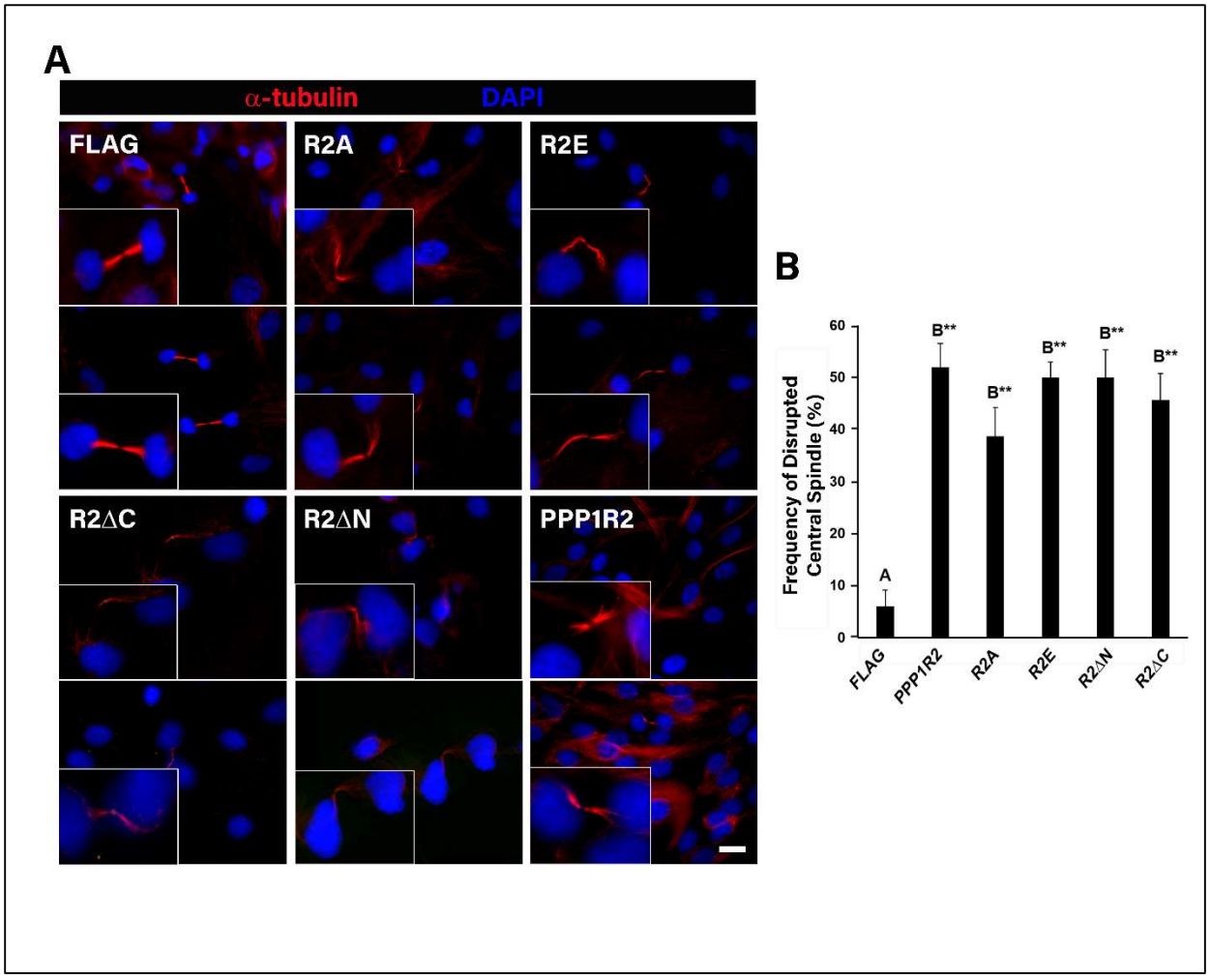
A
α-tubulin
DAPI
FLAG
R2A
R2E
R2ΔC
R2ΔN
PPP1R2
B
Frequency of Disrupted Central Spindle (%)
60
50
40
30
20
10
0
A
B**
B**
B**
B**
B**
FLAG
PPP1R2
R2A
R2E
R2ΔN
R2ΔC

Figure 2.8. PPP1R2 mutant overexpression increased frequency of disrupted central spindle structure. (A) ARPE-19 cells were transfected with FLAG, PPP1R2, and PPP1R2 mutants (R2A, R2E, R2ΔC, R2ΔN), fixed, and labeled with DAPI (blue) as well as α-tubulin (red) to stain the central spindle. Size bar equals 5μm. (B) Disrupted midbody frequency was quantified using Metamorph software and averages were calculated for three biological replicates (n>30 dividing cells). Statistically significant differences ($p \leq 0.05$) between experimental groups and empty vector control are indicated by differing letters above the bars and asterisks indicate *$p \leq 0.01$ and **$p \leq 0.001$.

D7. The Effect of PPP1R2 on PP1 and AURKA Activity at the Centrosome was Dependent on its C-terminus.

PPP1R2 overexpression stimulated PP1 activity, while at the same time reducing AURKA activity to undetectable levels (Figure 2.9A). These effects were independent of protein amount or phosphorylation state (Figure 2.9B). Overexpression of PPP1R2 truncated at its C-terminal domain (R2DC) did not change the activity of either PP1 or AURKA relative to control. In contrast, deletion of its N-terminus had the opposite effect by increasing PP1 activity to a level statistically similar to full length PPPR2 while at the same time abolishing AUKA, is necessary and sufficient to stimulate PP1 activity and inhibit AURKA activity.

Consistent with the observed changes in global enzyme activity after PPP1R2 overexpression, both AURKA and PP1 were less phosphorylated at the centrosome (Figure 2.9C-D) when PPP1R2 was overexpressed, corresponding to increased PP1 activity and reduced AURKA activity at the centrosome. This supports our proposal that PPP1R2 overexpression increased PP1 activity through its repression of AURKA activity resulting in a loss of phosphorylation at the centrosome. In addition, only PPP1R2 containing the C-terminus caused a decrease in pPP1 and pAURKA levels at the centrosome confirming that the C-terminus of PPP1R2 is required to inhibit AURKA and stimulate PP1 activity (Figure 2.9C-D).

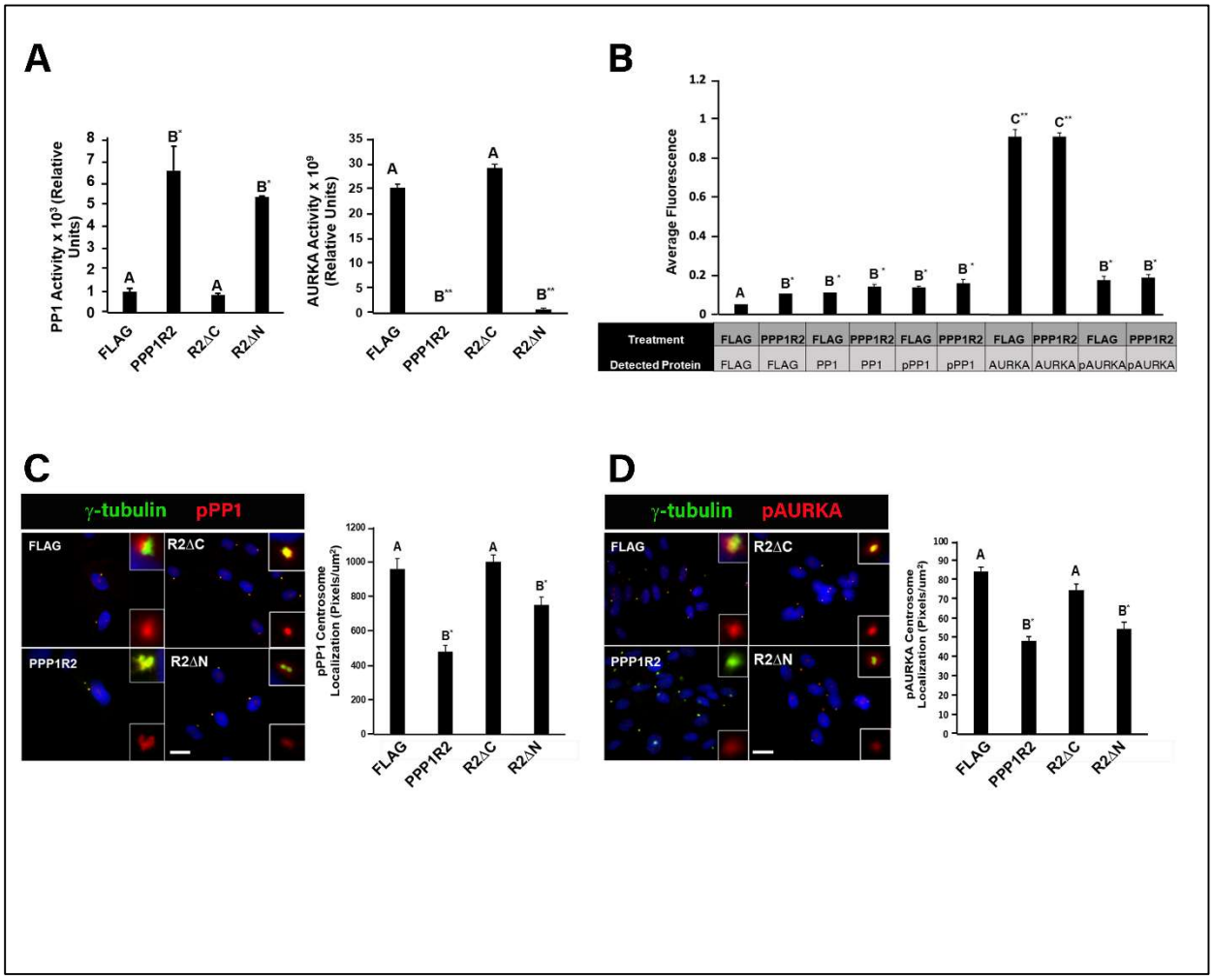
A
PP1 Activity x 10³ (Relative Units)
AURKA Activity x 10⁹ (Relative Units)
FLAG
PPP1R2
R2ΔC
R2ΔN
B
Average Fluorescence
Treatment
Detected Protein
FLAG PPP1R2 FLAG PPP1R2 FLAG PPP1R2 FLAG PPP1R2 FLAG PPP1R2
FLAG FLAG PP1 PP1 pPP1 pPP1 AURKA AURKA pAURKA pAURKA
C
γ-tubulin pPP1
pPP1 Centrosome Localization (Pixels/um²)
D
γ-tubulin pAURKA
pAURKA Centrosome Localization (Pixels/um²)

Figure 2.9. The effect of PPP1R2 on PP1 and AURKA activity at the centrosome was dependent on its C-terminus. (A) A graphical representation of the PP1 and AURKA activity of cells expressing each construct is shown. (B) Protein expression and phosphorylation levels of proteins indicated by the table below the graph were measured using ELISA. Cells were transfected with the indicated constructs, fixed, and labeled for γ-tubulin (red) and either (C) phosphorylated PP1 (pPP1, green) or (D) phosphorylated AURKA (pAURKA, green) antibodies. pPP1 and pAURKA levels at the centrosome were quantified using Metamorph software and are displayed as graphs in (C) and (D). Statistically significant differences ($p \leq 0.05$) between experimental groups and empty vector control are indicated by differing letters above the bars and asterisks indicate *$p \leq 0.01$ and **$p \leq 0.001$.

E. Discussion

AURKA regulates cell division, in part, through control of centrosome duplication, establishment of the bipolar spindle, and cytokinesis (Giet, R. et al., 2002; Glover, D. M. et al., 1995; Mangal, S., 2018). AURKA overexpression causes centrosome multiplication through failure of cytokinesis (Karthigeyan, D., 2011; Meraldi, P. et al., 2002; Nikonova, A. S., 2013; Zhou, H. et al., 1998). AURKA activity is opposed by PP1 through a negative feedback loop and this interaction is required for proper cell division. Disruption of binding of PP1 to AURKA leads to misalignment of chromosomes at metaphase (Katayama, H., 2001). The PP1 regulator PPP1R2 binds both AURKA and PP1 to positively and negatively regulate these enzymes (Satinover, D.L., 2006). These interactions are consistent with our results that truncation of either the PP1 or the AURK binding sites partially reduced the level of supernumerary centrosomes compared to the full-length PPP1R2 (Figure 2.2G).

Here I demonstrate that, like the negative feedback relationship between AURKA and PP1, PPP1R2 opposes the ability of AURKA to induce multiple centrosomes in cells. Our finding that PPP1R2 overexpression countered the effect of AURKA to increase centrosome number suggests that PPP1R2 inhibits AURKA; a finding that contradicts a previous study (Satinover, D. L., 2006). This difference may be a consequence of environment; our studies were conducted in live cells, while that study used recombinant proteins *in vitro* (Satinover, D. L., 2006) to show stimulation of AURKA activity by PPP1R2.

Although PPP1R2 overexpression with AURKA reduced centrosome number to control levels, it did not have the same effect when overexpressed with PP1. Instead, supernumerary centrosome frequency was reduced by about half in double transfectants compared to PP1 alone

(Figure 2.1J). I suspect this intermediate effect on centrosome number is due to an inhibition of PP1 by PPP1R2 to lessen phosphatase activity available to counteract AURKA. PPP1R2 is a substrate of PP1 (Bollen, M. et al., 2010); therefore, these two proteins could form a negative regulatory feedback loop when overexpressed in cells. Another explanation for this result is that PPP1R2 may regulate other proteins besides PP1 that oppose the effect of PP1 on centrosome number.

PP1 inhibits AURKA by dephosphorylating threonine 288 in the activation loop of AURKA (Katayama, H., 2001). As expected, co-overexpression of PP1 with AURKA reversed the accumulation of centrosomes seen when AURKA or PP1 were overexpressed individually (Figure 2.1J). Our results are consistent with a previous report that AURKA and PP1 activities are regulated by a negative feedback loop. These results provide critical support for previous findings of Katayama (Katayama, H., 2001), by demonstrating a biological consequence of this feedback loop.

The ability of PPP1R2 to inhibit PP1 phosphatase activity is dependent on the phosphorylation state of PPP1R2 [15, 36]. Glycogen synthase kinase-3β phosphorylates and inactivates PPP1R2, thereby activating PP1 (Cohen, 1989; Holmes, C. F., 1986; Sakashita, G. et al., 2003). Overexpression of the phosphomimetic mutant PPP1R2 R2E, but not the phosphonull mutant, caused supernumerary centrosomes to the same extent as wild-type PPP1R2 (Figure 2.2G) and approaching the increase seen with PP1 alone (Figure 2.1J). This is consistent with the inactivated phosphorylated form of PPP1R2 increasing activity of PP1 to increase centrosome number.

Increased expression of AURKA causes tetraploidization due to failed cytokinesis (Meraldi, P. et al., 2002). Likewise, increased expression of PPP1R2 caused increased cellular DNA content. One possibility for this result is that PPP1R2 participates in cytokinesis. PPP1R2's role in cytokinesis is supported by the finding that PPP1R2 is found at the midbody (Figure 2.4A, C) and that overexpression of its phosphomimetic as well as truncation mutants lengthened the central spindle and disrupted its structure (Figure 2.6-7). Given that PPP1R2 interacts with both PP1 and AURKA, and has been reported to balance Aurora B and PP1 activity during cytokinesis, I propose that PPP1R2 coordinates the activity of kinases and PP1 to maintain central spindle architecture (McKenzie, C. et al., 2016; Satinover, D. L. et al., 2006; Wang, W. et al., 2008).

I demonstrate that both PPP1R2 and PP1 localized to the midbody and that PP1's localization there is dependent on both N- and C-terminal domains of PPP1R2 (Figure 2.4C). Notably, full length PPP1R2 enhanced PP1 localization at the midbody. The phosphomimetic mutant also increased PP1 localization supporting a role for PPP1R2 phosphorylation in targeting PP1 at cytokinesis. These data suggest that PPP1R2 targets PP1 to the midbody and this action relies on PPP1R2 phosphorylation. I propose that overexpression of PPP1R2 leads to an increase in phosphatase activity at the midbody resulting in less protein phosphorylation at the midbody with serious consequences to its function, as I observe here. During preparation of this manuscript, a study was published reporting the midbody interactome [38]. Results reported here expands the PP1 interactome to include PPP1R2. Further studies will be necessary to uncover the molecular mechanisms of PPP1R2's effect on midbody structure through changes in activity of PP1 and AURKA at the centrosome.

PP1 regulates both phosphorylation of central spindle complexes and cytokinesis by establishing proper recruitment of Cep55 and related abscission machinery to the midbody ring (Gao, K. et al., 2018). Aurora B Kinase (AURKB) has been shown to regulate midbody architecture and PPP1R2 has been shown to regulate AURKB at the midbody (Mckenzie, C. et al., 2016; Wang, W., Stukenberg, P. T., & Brautigan, D. L., 2008). Our data further demonstrates misshaped and elongated midbody structures during overexpression of both PPP1R2 phosphomimetic and truncation mutants (Figure 2.6-7). Based on our data and previous reports regarding Aurora B kinase midbody regulation I propose PPP1R2 has a role in regulating kinase and phosphatase activity to control central spindle architecture and therefore cytokinesis (Mckenzie, C. et al, 2016; Wang, W., Stukenberg, P. T., & Brautigan, D. L., 2008). The mechanism whereby PPP1R2's recruitment of PP1 to the midbody is involved in maintaining midbody architecture will be investigated in future studies.

PPP1R2 overexpression reduced phosphorylation of both AURKA and PP1 at the centrosome (Figure 2.8C-D) independent of a change in global phosphorylation of either enzyme (Figure 2.8B). These changes correlated with an increase in PP1 activity and a decrease in AURKA activity (Figure 2.8A). Inhibition of AURKA by the C-terminus of PPP1R2 caused a stimulation of PP1 activity and a subsequent loss of AURKA and PP1 phosphorylation level at the centrosome (Figure 2.8A). This is consistent with our proposal that inactivation of AURKA by PPP1R2 results in activation of PP1 at the centrosome. Changes in phosphorylation at the centrosome could have multiple effects on centrosome function including impaired protein recruitment and maintenance of the pericentriolar matrix (Meng, L. et al, 2015; Nasa, I. et al, 2017; Zhou, H. et al, 1998). Further

studies will be necessary to investigate PPP1R2's role in regulating the structure of the pericentriolar matrix and protein recruitment.

F. Conclusions

Our data support the conclusion that PPP1R2 represents a new regulator of cytokinesis. This is consistent with both PPP1R2's localization at the midbody and the increase in nuclear content when PPP1R2 is overexpressed. PPP1R2 overexpression increased PP1 localization at the midbody and this recruitment required both terminal domains on PPP1R2 necessary for binding to AURKA and PP1. Furthermore, both phosphorylation site and truncation mutants of PPP1R2 caused disruption in central spindle structure, both in length and integrity. I conclude that the increase in centrosome numbers I see after PPP1R2 overexpression is the result of incomplete cytokinesis caused by increased phosphatase activity at the midbody. In addition, I show that PPP1R2 interacts with AURKA and PP1 to regulate phosphorylation and activity of both AURKA and PP1 at the centrosome. Paradoxically, I find that overexpression of PPP1R2 increased PP1 activity rather than its well documented role as an inhibitor. Our study demonstrates that PPP1R2 activation of PP1 was dependent on PPP1R2's C-terminus; the binding site for AURKA. Therefore, I propose that the increase in PP1 activity I observe is the indirect result of decreased activity of AURKA, an inhibitor of PP1 in PPP1R2 overexpressing cells. Increased phosphatase activity at the midbody would alter the mechanics of midbody assembly and abscission at cytokinesis.

CHAPTER III: THE PP1 REGULATOR PPP1R2 REGULATES CENTROSOME PROTEIN RECRIUTMENT, CENTROSOME ENZYME PHOSPHORYLATION LEVELS, AND MICROTUBULE NUCLEATION

A. Summary

Background: Protein recruitment is critical for the maturation of the centrosome's PCM (1999; Kim, S. & Rhee, K., 2014; Meraldi, P., & Nigg, E., A., 2002; Woodruff J. B. et al., 2014, Woodruff J. B. et al., 2015). Pericentrin and Cep192 recruitment is critical for enzymes and microtubule nucleation complexes critical for mitotic spindle assembly to bind at the centrosome (Dictenberg, J. B. et al., 1998; Farache, D. et al. 2018; Gomez-Ferreria, M. A., et al., 2007; Gomez-Ferreria, M. A., & Sharp, 2008; Joukov, V., Walter, & De Nicolo, A., 2014; Nasa, I., 2017). Phosphorylation modulates enzyme activity and protein affinity to maintain proper protein recruitment at the centrosome (Joukov, V. et al., 2014; Lee, K., & Rhee, K., 2011; Meraldi, P., & Nigg, E. A., 2002).

Cep192 is responsible for recruitment of AURKA, PP1, and Plk1 during centrosome maturation, and this recruitment is essential for γ-tubulin complex formation at the PCM (Joukov, V. et al., 2014; Nasa, I. et al., 2017). Pericentrin forms complexes with γ-tubulin to facilitate microtubule nucleation (Dictenberg, J. B. et al., 1998; Gomez-Ferreria, M. A. et al., 2012; Joukov, V. et al., 2014; Laurence, H. et al., 2009; Nasa, I. et al., 2017; Pinyol, R. et al., 2013). Despite what is known about these mechanisms, it remains unclear how PP1, Plk1, and AURKA interact to modulate protein phosphorylation at the centrosome. The results described here support a novel mechanism whereby phosphoprotein protein phosphatase 1 regulatory subunit 2 (PPP1R2), a subunit of PP1, alters phosphorylation of AURKA, PP1, and Plk1 to maintain γ-tubulin and pericentrin at the centrosome. In addition, evidence is provided for PPP1R2 in regulating microtubule nucleation at the centrosome.

Results: Overexpression of PPP1R2, AURKA, and PP1 induced defects in γ-tubulin and pericentrin recruitment to the centrosome. This is supported by PPP1R2, AURKA, and PP1 overexpression resulting in abnormal localization of γ-tubulin to the cytoplasm. PPP1R2 regulates centrosome protein recruitment differently when overexpressed with either AURKA or PP1. Co-overexpression of PPP1R2 with AURKA restored γ-tubulin localization to the centrosome while co-overexpression of PPP1R2 and PP1 increased γ-tubulin mislocalization.

PPP1R2 interacts with PP1 primarily at its N-terminal and has C-terminal residues that specifically interact with AURKA. Overexpression of PPP1R2 truncation mutants further expanded on PPP1R2's interaction with AURKA and PP1 during centrosome protein recruitment. Overexpression of both N- and C-terminal PPP1R2 truncation mutants significantly increased γ-tubulin mislocalization. PPP1R2's regulation of centrosome protein recruitment was dependent on its phosphorylation state. This is seen during overexpression of either phospho-mimetic or phospho-null PPP1R2 mutants which restored γ-tubulin localization at the centrosome.

PPP1R2 overexpression also lowered phosphorylation levels at the centrosome. This is supported by PPP1R2 significantly lowering pPlk1 levels at the centrosome. Finally, a nocodazole washout assay determined whether PPP1R2 overexpression's perturbation of centrosome protein recruitment also disturbed microtubule nucleation. Following microtubule depolymerization by nocodazole, PPP1R2 and PPP1R2 phosphomimetic overexpression resulted in a suppression of microtubule nucleation recovery at the centrosome.

Conclusions: Findings from this study demonstrate a role for PPP1R2 in regulating protein recruitment through changes in centrosome enzyme phosphorylation. PPP1R2's regulation of protein recruitment maintains PCM stability. This is consistent with PPP1R2, AURKA, and PP1 overexpression

significantly increasing the cytoplasmic localization of γ-tubulin and pericentrin. Additionally, PPP1R2 overexpression reduced phosphorylation levels of AURKA, PP1, and Plk1 at the centrosome. PPP1R2 overexpression also suppressed microtubule nucleation. A model is proposed whereby PPP1R2 overexpression suppresses microtubule nucleation by causing PCM instability.

B. Introduction

The PCM undergoes radical restructuring throughout the cell cycle (Conduit, P. T. et al., 2010; Conduit, P., Wainman, A., & Raff, J. W., 2015; Lüders, J., 2012; Mattison, C. P., & Winey, M., 2006; Nigg ,E. A., & Stearns, T., 2011; Woodruff, J. B. et al., 2015). This restructuring happens through a series of molecular events termed the centrosome cycle (Meraldi, P., & Nigg, E. A., 2002). The centrosome cycle is tightly coupled to the cell cycle, and consists of the following steps associated with the corresponding cell cycle phase: centrosome disengagement/G_1, centrosome duplication/S, centrosome maturation and separation /G_2, and mitotic spindle assembly/M. Centrosome maturation begins at the S/G_2 transition of the cell cycle and continues until the end of G_2 (Meraldi, P., & Nigg, E. A., 2002; Piehl, M. et al., 2004). Cell cycle regulators can have overlapping targets at the centrosome and link the centrosome cycle to the cell cycle (Vandré, D. D. et al., 2000). Results from previous reports correlate dysregulated centrosome cycles with abnormal ploidy levels and genomic instability (Yaguchi, K., et al., 2018). The centrosome matures by both procentriole elongation and the recruitment of essential proteins to its pericentriolar matrix (Lüders, J., 2012; Palazzo, A. F. et al., 2000; Winey, M., & O'Toole, E., 2014; Woodruff, J. B. et al., 2014). The recruitment of these proteins enables the centrosome's PCM to become a stable platform for microtubule nucleation and organization (Joukov, V., & De Nicolo, A., 2018; Lüders, J., 2012; Palazzo, A. F. et al., 2000; Piehl, M., et al., 2004; Woodruff, J. B. et al., 2014; Woodruff, J. B. et al., 2015). Fully mature centrosomes separate and migrate to opposite poles of the cell to establish the bipolar spindle. The centrosome becomes fully mature when two types of proteins are recruited, specifically scaffolding

(Conduit, P. T. et al., 20d10; Dammermann, A., & Merdes, A., 2002; Delaval, B., & Doxsey, S. J., 2010; Dictenberg, J. B. et al., 1998; Gomez-Ferreria, M. A. et al., 2007; Gopalakrishnan, J. et al., 2011; Joukov, V. et al., 2014; Lüders, J., 2012; Takahashi, M., Yamagiwa, A., Nishimura, T., Mukai, H., & Ono, Y., 2002) and microtubule-nucleating proteins (Farache, D. et al., 2018; Oakley, B. R. et al., 2015; Tovey, C. A., & Conduit, P. T., 2018).

PCM scaffolding proteins provide a stable structure for protein recruitment necessary for microtubule nucleation complex assembly at the centrosome. Pericentrin is a large scaffolding protein that spans the entirety of the PCM's structure and maintains the stability of the PCM (Dammermann, A., & Merdes, A., 2002; Delaval, B., & Doxsey, S. J., 2010; Lee, K., & Rhee, K., 2011). Pericentrin is the central protein for Cep192 recruitment and γ-tubulin ring complex formation. Cep192 is recruited to pericentrin during the G_2/S phase transition and brings AURKA, PP1, and Plk1 to the centrosome through a series of phosphorylation events (Joukov, V., & De Nicolo, A., 2018). Centrosome maturation begins when Cep192 recruits Plk1 and AURKA to the centrosome (Joukov, V., & De Nicolo, A., 2018). The phosphorylation events begin when AURKA autophosphorylates at T295/T288 within its activation loop. Activated AURKA then phosphorylates Plk1 at T201/T210, resulting in Plk1 activation. Activated Plk1 then phosphorylates Cep192 at T46/T44, resulting in the recruitment of additional Plk1 (Joukov, V. et al., 2014). Cep192 is structurally altered by these phosphorylation events and can then interact with Nedd1 that is complexed with γ-tubulin rings (Joukov, V., & De Nicolo, A., 2018). Overall, the microtubule nucleating complexes assembled during centrosome maturation prepare the centrosome for mitotic spindle assembly (Farache, D. et al., 2018; Khodjakov, A., & Rieder, C. L., 1999; Lee, K., & Rhee, K., 2011; Oakley, B. R. et al., 2015; Palazzo, R. E. et al., 2000; Petretti, C. et al., 2006; Tovey, C. A., & Conduit, P. T., 2018).

PP1 is recruited to the centrosome by Cep192 during centrosome maturation, but it remains unknown how PP1 activity regulates protein recruitment during centrosome maturation (Nasa, I. et al., 2017). It is also unknown if PPP1R2 regulates centrosome maturation because of its established role as a

regulator of two critical centrosome enzymes: AURKA and PP1 (Eto, M., Elliott, E., Prickett, T. D., & Brautigan, D. L., 2002; Li, M., Satinover, D. L., & Brautigan, D. L., 2007; Lukasiewicz, K. B., & Lingle, W. L., 2009; Nasa, I., et al., 2017; Satinover, D. L., Leach, Stukenberg, P. T., & Brautigan, D. L., 2004). The overall aim of this work was to evaluate the effects of PPP1R2 overexpression on centrosome protein localization and function as well as enzyme activity at the centrosome. In this chapter, I investigated the results of PPP1R2 overexpression in disruption of Cep192 associated γ-tubulin recruitment as well as pericentrin localization at the centrosome. I shifted the investigation from cytokinesis to centrosome maturation because PPP1R2 has been shown to impact AURKA and PP1 activity as previously reported (Li, M. et al., 2007; Satinover D. L., 2004). My findings in Chapter II corroborate this report as I show PPP1R2 overexpression activated PP1 and inactivated AURKA.

Microtubules carry out the majority of the centrosome's function through their dynamic instability. Microtubule dynamic instability is the assembly and eventual rapid disassembly, termed catastrophe, of microtubule tubulin dimer complexes. GTP-bound tubulin forms dimers which polymerize into straight protofilaments that are highly stable. In contrast, GDP-bound tubulin forms curved protofilaments which are highly unstable and favor depolymerization (Howard, J. & Hyman, A. A., 2003; Hyman, A. A. et al., 1995; Melki, R. et al., 1989). Both GTP- and GDP-bound tubulin are incorporated into the microtubule structure, and it is the GTP-bound tubulin that confers structural stability to prevent microtubule catastrophe (Howard J., & Hyman, A. A., 2003).

Both β-tubulin and α-tubulin participate in maintaining GTP and GDP levels in the microtubule's structure to regulate microtubule catastrophe. β-tubulin acts as its own GTPase stimulating protein (by hydrolyzing GTP and reducing GTP levels in the microtubule. GTP-hydrolysis by β -tubulin in the microtubule is slow allowing the microtubule to polymerize (Desai, A., & Mitchison, T. J., 1997; Howard, J., & Hyman, A. A., 2003 ; Hyman, A. A., et al., 1995; Müller-Reichert, T., Chrétien, D., Severin, F., & Hyman, A. A., 1998). α-tubulin is constitutively bound to GTP and inherently increases GTP levels in the

microtubule during microtubule polymerization (Desai A., & Mitchison, T. J., 1997; Howard, J., & Hyman, A. A., 2003; Spiegelman, B. M., 1977). Once GTP-bound tubulin is hydrolyzed by β-tubulin, the microtubule's curvature increases and destabilizes the latticework of the microtubule, resulting in catastrophe. Microtubule catastrophe begins predominately at the positive end of the microtubule, as β-tubulin is localized at the positive end of the αβ dimer and establishes the microtubule's polarity. GAP activity at the plus end of the microtubule increases the rate of GTP hydrolysis, resulting in the exposure of GDP-bound tubulin and microtubule catastrophe. (Tian, G., Bhamidipati, A., Cowan, N. J., & Lewis, S. A., 1999) (Figure 3.1). There are situations where GTPases increase the rate of microtubule polymerization by opposing GAP activity at the microtubule. The mechanism primarily responsible for increasing microtubule GTP hydrolysis remains unclear, however reports implicate multiple pathways (Bowne-Anderson, H., Zanic, M., Kauer, M., & Howard, J., 2013; Coombes, C. E., Yamamoto, A., Kenzie, M. R., Odde, D. J., & Gardner, M. K., 2013; Gardner, M. K. et al., 2011; Goodson, H. V., & Jonasson, E. M., 2018; Grishchuk, E. L., Molodtsov, M. I., Ataullakhanov, F. I., & McIntosh, J. R., 2005; Howard, J., & Hyman, A. A., 2009; Margolin, G., et al., 2012; Tian, G., et al., 1999).

Both microtubule dynamic instability and the anchoring of microtubule minus ends to the centrosome are critical for mitotic spindle assembly and function. The mitotic spindle is a specialized microtubule structure that both captures and aligns chromosomes during mitosis. γ-tubulin is a protein which forms concentric ring complexes that allow stable nucleation of microtubules from their negative ends at the centrosome. Microtubule negative end stability is an essential part of the mitotic spindle's structure and allows the centrosome to organize and maintain both mitotic spindle assembly and size during mitosis (Greenan, G. et al., 2010; Hoffmann, I., 2020; Keller, L. C., Wemmer, K. A., & Marshall, W. F., 2010; Meraldi, P., 2016). The anchoring of the mitotic spindle to the centrosome allows microtubule positive ends to extend and retract to interact with kinetochore complexes at the chromosome (Bakhoum,

S. F., Thompson, S. L., Manning, A. L., & Compton, D. A., 2009; Horio, T., & Murata, T., 2014; Kirschner, M., & Mitchison, T., 1986).

Microtubule end capping and tension are essential for proper chromosome segregation during mitosis (Bakhoum, Thompson, Manning, & Compton, 2009). Microtubules become stable once they contact the kinetochore and anchor the chromosome to the mitotic spindle (Bakhoum, S. F., et al., 2009). The chromosomes are captured by the mitotic spindle once the microtubules-kinetechore structure is further stabilized by positive end microtubule capping as well as structural tension (Andrews, P. D., Ovechkina, Y., Morrice, N., Wagenbach, M., Duncan, K., Wordeman, L., et al., 2004; Infante, A. S., Stein, M. S., Zhai, Y., Borisy, G. G., & Gundersen, G. G., 2000; Palazzo, A., Cook, T., Alberts, A., & Gundersen, G, 2001; Tirnauer, Canman, Salmon, & Mitchison, 2002) Microtubule end capping and kinetochore attachment is regulated by both Rho-GTPases as well as EB1 positive end tracking proteins (Infante, A. S., Stein, M. S., Zhai, Y., Borisy, G. G., & Gundersen, G. G., 2000; Palazzo, A., Cook, T., Alberts, A., & Gundersen, G, 2001; Tirnauer, Canman, Salmon, & Mitchison, 2002). (Andrews, P. D., Ovechkina, Y., Morrice, N., Wagenbach, M., Duncan, K., Wordeman, L., et al., 2004).

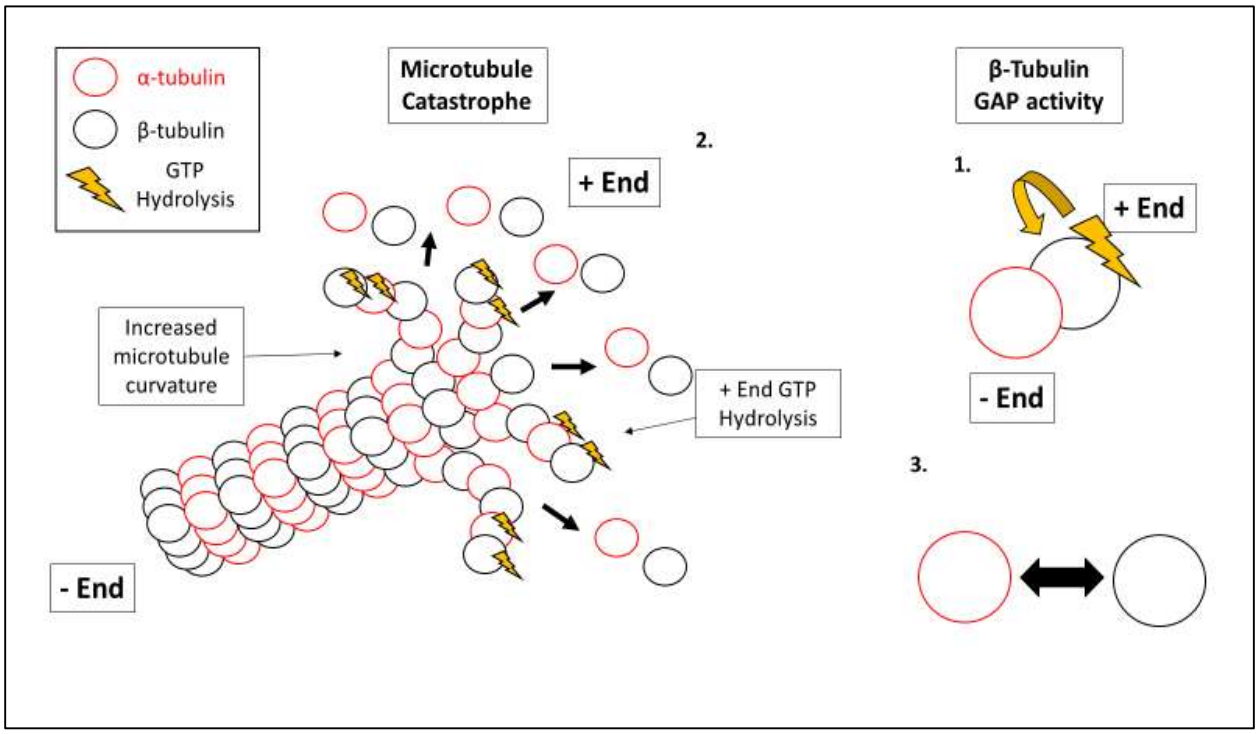
α-tubulin
β-tubulin
GTP Hydrolysis
Microtubule Catastrophe
2.
+ End
Increased microtubule curvature
+ End GTP Hydrolysis
- End
β-Tubulin GAP activity
1.
+ End
- End
3.

Figure 3.1. Schematic depicting microtubule catastrophe and β-tubulin GTPase activating protein activity. 1. First β-tubulin hydrolyzes GTP and 2. then the microtubule protofilaments begin to dissociate. 3. Finally, the tubulin hetherodimers dissociate leading to rapid depolymerization beginning at the microtubule‘’s plus

C. Experimental Procedures

C1. Cell Culture and DNA Transfection

Human pigmented retinal epithelial cells (ARPE-19; American Type Tissue Collection) were grown in DMEM-F12 media supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. The PPP1R2 overexpressing plasmid was constructed by inserting the *Ppp1R2* coding sequence (a gift of Dr. Srinivasan Vijayaraghavan, Kent State University) in-frame with the FLAG tag of the mammalian expression vector CMVFLAG 3X-14 (Sigma Aldrich). The PP1 plasmid was a gift of Dr. James McDonald, Western University, Cancer Research Center. The AURKA plasmid was obtained from Dr. Eric Nigg, University of Basil. PPP1R2 phospho-mutants were generated using the QuikChange® site-directed mutagenesis kit (Stratagene). ARPE-19 cells plated on glass coverslips were grown to approximately 70% confluence then grown for 24 hours prior to transfection. Cells were transfected using Lipofectamine-2000 (Invitrogen) according to manufacturer's recommendations.

C2. Immunofluorescence and Measurement of Protein Localization.

Transfected cells were fixed and permeabilized with methanol, then nonspecific binding was blocked by incubation in 3% BSA in TBST (20 mM Tris, pH 7.5, 150 mM NaCl, 2 mM EGTA, 0.1% Triton X-100) for 30 minutes. The cells were incubated overnight at 4°C with primary antibody, then with secondary antibodies conjugated to Alexa Fluor-488 and -594 (1:200, Life Technologies). α-tubulin was detected with a goat polyclonal antibody (1:200, 74010 clone TUBA4A, Life Sciences), FLAG with a rabbit polyclonal antibody (1:500, PA1984B; Thermo Fisher Scientific), and γ-tubulin with a mouse monoclonal antibody (1:50, PA5-34815, Thermo Fisher Scientific). DNA was labeled with Vectashield (Vector Laboratories) mounting media containing 4′, 6-diamidino-2-phenylindole (DAPI) dye.

The intracellular localization of proteins was visualized using a Nikon E600 fluorescence microscope, Pan Fluor 100X objective (N.A. 0.5-1.3) or Pan Fluor 40X objective (N.A. 0.75), fit with

appropriate filters. Images were captured with an Orca II CCD camera (Hamamatsu) and Metamorph image analysis and acquisition software (Universal Imaging Corporation). Images were exported to ImageJ (NIH) and only linear adjustments to brightness and/or contrast were performed.

γ-tubulin intensity immediately around the centrosome was subtracted from the total intensity within the cell. The total integrated intensity of cytoplasmic γ-tubulin within the cell was divided by the area (μm^2) of the cell to normalize values between cells of different sizes.

C3. Nocodazole Washout Assay

ARPE-19 cells (ARPE-19; American Type Tissue Collection) were treated with 10 μM nocodazole for one hour at 37°C. Cells were washed three times with 1X PBS after nocodazole incubation and placed in fresh DMEM F12 media. Cells were allowed to recover from nocodazole treatment for 5 minutes then cells collected and fixed over a 5-minute time period. Collection times included 0, 3, 4, and 5 minutes following nocodazole washout. Cells were collected and fixed according to the immunofluorescent protocol described in section C1. Metamorph software was used to measured α-tubulin intensity within a 1 μm^2 area around the centrosome to monitor microtubule regrowth over the time course of the experiment.

C4. Statistical Analyses

The data for centrosome quantitation was expressed as mean ± SEM. The differences between groups were analyzed using a one-way ANOVA and unpaired Student's t-test with JMP Version 13.1. Differences at $p \leq 0.05$ were considered statistically significant. I used the software Q*Power to calculate sample sizes appropriate for 80% power and an alpha value <0.2.

D. Results

D1. PPP1R2 Overexpression Results in PCM instability

To assess how PPP1R2 regulates centrosome function I first overexpressed PPP1R2 and found that cells had increased levels of mislocalized γ-tubulin compared to controls (Figure 3.1A-B). γ-tubulin is typically localized as perinuclear puncta. This is a classic presentation of centrosome localization (Raynaud-Messina, B., & Merdes, A., 2007; Tovey, C. A., & Conduit, P. T., 2018). However, γ-tubulin showed a significant shift in localization from its normal perinuclear site to the cytosol as well as the nucleus ($p<0.01$) in cells overexpressing PPP1R2 compared to controls (Figure 3.1A-B, H).

I next investigated the effect of AURKA and PP1 overexpression on γ-tubulin localization. Overexpression of either AURKA or PP1 resulted in γ-tubulin mislocalization similar to that seen in PPP1R2 overexpressing cells (Figure 3.1C-D,H). In order to further investigate how PPP1R2 interacts with AURKA and PP1 in centrosome regulation I co-overoverxpressed PPP1R2 with AURKA and PP1. PPP1R2/AURKA co-overexpression recovered γ-tubulin localization to the centrosome (Figure 3.1E,H). PPP1R2/PP1 co-overexpression increased γ-tubulin cytosolic localization compared to controls, suggesting PPP1R2 regulates centrosome maturation through PP1 (Figure 3.1G,H). As expected, co-overexpression of AURKA with its known antagonist PP1 restored γ-tubulin centrosome localization (Figure 3.1F,H).

Pericentrin is a central scaffolding protein for the PCM (Delaval, B., & Doxsey, S. J., 2010). Because of the observed relocalization of γ-tubulin, I assessed pericentrin localization to further investigate PPP1R2's effects on PCM stability. Pericentrin was also found dispersed in the cytosol of cells overexpressing PPP1R2 compared to controls (Figure 3.1I-J).

D2. PPP1R2 Interacts with both AURKA and PP1 to Regulate γ-tubulin Centrosome Localization

To investigate the mechanism by which PPP1R2 regulates γ-tubulin recruitment, I used both truncation and site-directed mutants of PPP1R2 described previously in Chapter II (Figure 2.3A). Briefly,

the truncation mutants were designed to interfere with PPP1R2's interaction between PP1, at its N-terminus, and AURKA, at its C-terminus, to investigate how PPP1R2's interaction with PP1 and AURKA compares in centrosome regulation. PPP1R2 phosphomutants altered PPP1R2's phosphorylation site at Thr72 to determine whether PPP1R2's regulation of the centrosome was dependent on its phosphorylation state. Overexpression of both phospho-mimetic (R2E) and phospho-null (R2A) mutants resulted in a restoration of γ-tubulin localization (Figure 3.2C-D,G) This suggests that phosphorylation of PPP1R2 at Thr72 is necessary to maintain γ-tubulin centrosome recruitment. Overexpression of both the N- and C-terminal PPP1R2 truncation mutants resulted in significant ($p<0.01$) γ-tubulin mislocalization (Figure 3.2E-F,G). Given that PPP1R2's N-terminus is primarily responsible for its interaction with PP1 and its C-terminus with AURKA, these data confirm that PPP1R2 interacts with both enzymes to regulate γ-tubulin recruitment. The localization of γ-tubulin was quantified using Metamorph software for all treatments (Figure 3.2G).

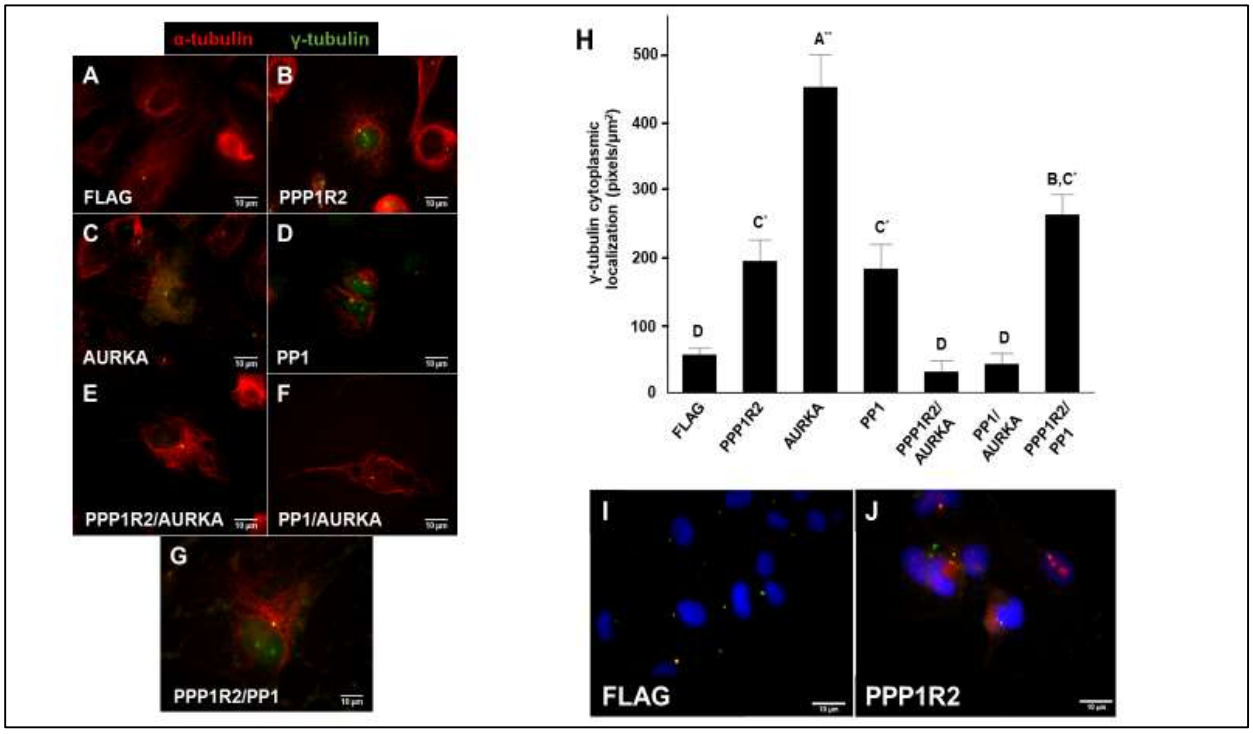
α-tubulin
γ-tubulin
A
FLAG
B
PPP1R2
C
AURKA
D
PP1
E
PPP1R2/AURKA
F
PP1/AURKA
G
PPP1R2/PP1
H
γ-tubulin cytoplasmic localization (pixels/μm²)
500
400
300
200
100
0
A**
C*
C*
D
D
D
B,C*
FLAG
PPP1R2
AURKA
PP1
PPP1R2/ AURKA
PP1/ AURKA
PPP1R2/ PP1
I
FLAG
J
PPP1R2

Figure 3.2. PPP1R2 affects centrosome γ-tubulin localization through interaction with AURKA and PP1. (A-G) ARPE-19 cells were transfected either singly or in combination with plasmids expressing PPP1R2, AURKA, PP1 and empty vector as control (FLAG). Transfected cells were stained for γ-tubulin (green) and α-tubulin (red). (H) γ-tubulin cytoplasmic localization was quantified in a minimum of 100 cells for each treatment group in three replicates. Size bars equal 10 μm. (H) Graphical representation of γ-tubulin cytoplasmic localization in cells transfected with each of the indicated plasmids individually or in combination. Statistically significant differences ($p<0.05$) between groups are indicated by differing letter notations above the bars and error bars represent standard error of the mean. Statistically significant differences are indicated with asterisks (*=$p\leq0.01$, **=$p\leq0.001$) compared to control. (I-J) Transfected cells were stained for γ-tubulin (green), pericentrin (red) and DAPI (blue).

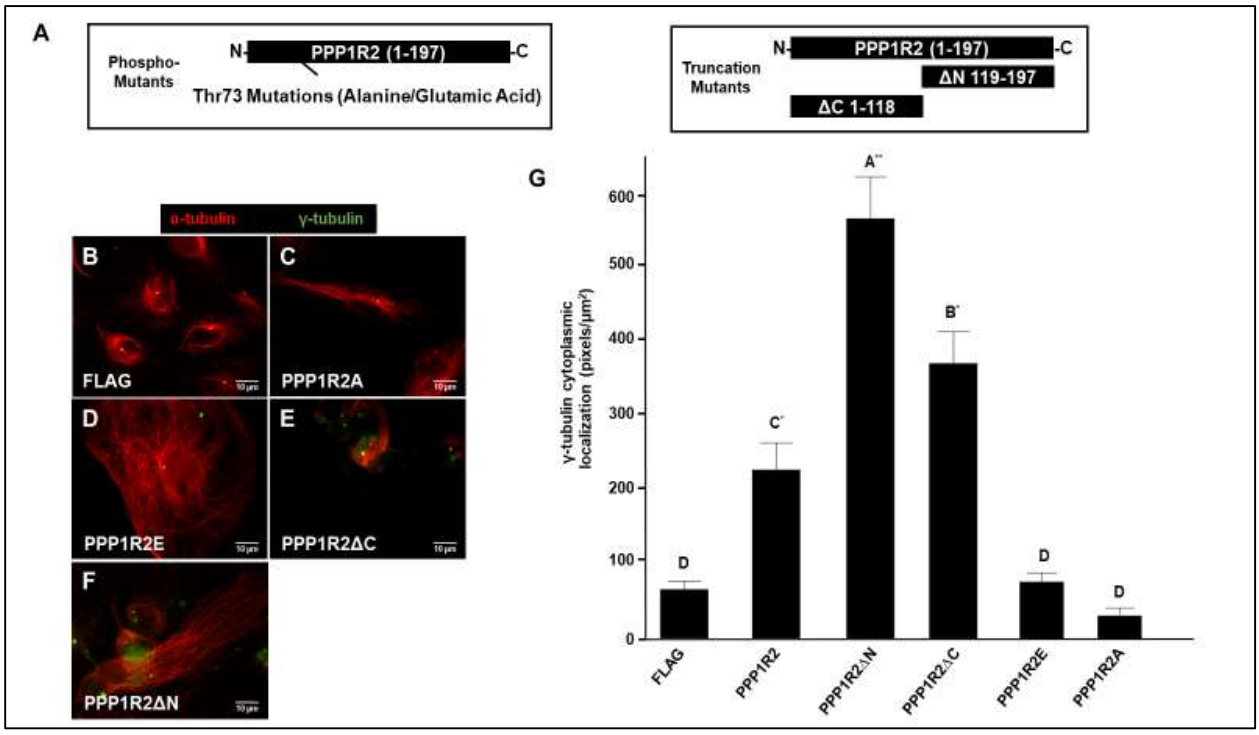
A
Phospho-Mutants
N-
PPP1R2 (1-197)
-C
Thr73 Mutations (Alanine/Glutamic Acid)
Truncation Mutants
N-
PPP1R2 (1-197)
-C
ΔN 119-197
ΔC 1-118
α-tubulin
γ-tubulin
B
FLAG
C
PPP1R2A
D
PPP1R2E
E
PPP1R2ΔC
F
PPP1R2ΔN
G
γ-tubulin cytoplasmic localization (pixels/μm²)
600
500
400
300
200
100
0
A**
B*
C*
D
D
D
FLAG
PPP1R2
PPP1R2ΔN
PPP1R2ΔC
PPP1R2E
PPP1R2A

Figure 3.3. PPP1R2 interaction with both PP1 and AURKA affects centrosome γ-tubulin localization and is phosphorylation dependent. (A) Schematics of the PPP1R2 mutants used for transfection. The left schematic shows the position of phosphorylation site mutants involving the Thr73 residue including both the threonine to alanine phosphonull mutation PPP1R2A (R2A) and threonine to glutamic acid

D3. PPP1R2 Overexpression Inhibits Microtubule Nucleation

Pericentrin and γ-tubulin ring complex recruitment to the PCM forms essential anchoring points for microtubule nucleation. Since PPP1R2 overexpression caused mislocalization of both γ-tubulin and pericentrin, I next investigated if this disruption of the PCM also affected the centrosome's ability to nucleate microtubules. Cells were treated with a nocodazole washout procedure with and without PPP1R2 overexpression. Nocodazole was used to depolymerize microtubules and then removed from the media to allow microtubules to polymerize to form an aster. The amount of microtubule nucleation after the removal of nocodazole in cells expressing each vector was evaluated by measuring α-tubulin levels within a 1 μm^2 area around the centrosome. In control cells, aster formation occurred by 3 minutes, with robust nucleation recovery after 4 minutes (Figure 3.3A-C). PPP1R2 overexpression significantly inhibited microtubule nucleation recovery over time compared to controls ($p<0.01$) (Figure 3.3G). Aster formation did not occur in PPP1R2-overexpressing cells over the 5-minute time course (Figure 3.3D-E).

PPP1R2 phospho-mutants were then utilized to investigate whether PPP1R2's effect on microtubule nucleation depended on its phosphorylation state (Figure 3.4). Overexpression of only the PPP1R2 phospho-mimetic mutant inhibited microtubule nucleation recovery (Figure 3.4G-I panel, M). Overall, these data suggest that PPP1R2 regulates microtubule nucleation and that this regulation is dependent on its phosphorylation at Thr72.

D4. PPP1R2 Overexpression Reduces pPlk1 Localization at the Centrosome.

AURKA, PP1, and Plk1 form a complex with Cep192 during centrosome maturation (Joukov, V. et al., 2014; Nasa, I., et al., 2017). In Chapter II, I demonstrated that PPP1R2 overexpression resulted in lower phosphorylation of both PP1 and AURKA at the centrosome. In addition, PPP1R2 overexpression inactivated AURKA, which activates Plk1 during centrosome maturation (Joukov, V. et al., 2014). Since AURKA phosphorylates and activates Plk1 at the centrosome, I next investigated whether PPP1R2

overexpression affected the level of phosphorylated Plk1 (phospho-Plk1) (Joukov, V. et al., 2014). PPP1R2 overexpression significantly reduced phospho-Plk1 at the centrosome (Figure 3.5C-E, $p<0.01$). This is consistent with findings that PPP1R2 overexpression inactivates AURKA as AURKA activity is essential for Plk1 phosphorylation at the centrosome Altogether, these data suggest a novel role for PPP1R2 regulation of Plk1 at the centrosome.

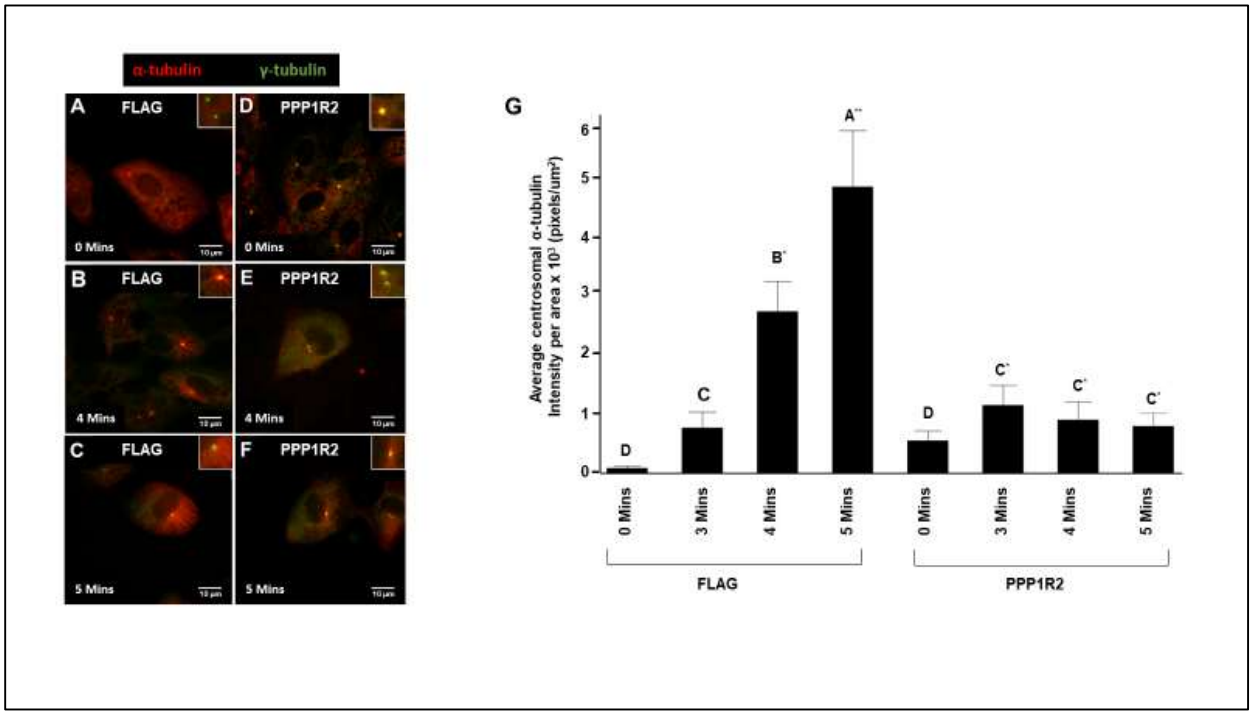
α-tubulin
γ-tubulin
A FLAG
0 Mins
B FLAG
4 Mins
C FLAG
5 Mins
D PPP1R2
0 Mins
E PPP1R2
4 Mins
F PPP1R2
5 Mins
G
Average centrosomal α-tubulin Intensity per area x 10³ (pixels/um²)
0
1
2
3
4
5
6
D
C
B*
A**
D
C*
C*
C*
0 Mins
3 Mins
4 Mins
5 Mins
0 Mins
3 Mins
4 Mins
5 Mins
FLAG
PPP1R2

Figure 3.4. PPP1R2 suppresses microtubule nucleation recovery at the centrosome. (A-F) ARPE-19 cells were treated with nocodazole to depolymerize microtubules. Transfected cells were allowed to recover microtubule nucleation at the centrosome after nocodazole washout over the indicated time course. Transfected cells were stained for γ-tubulin (green) and α-tubulin (red). (G) α-tubulin

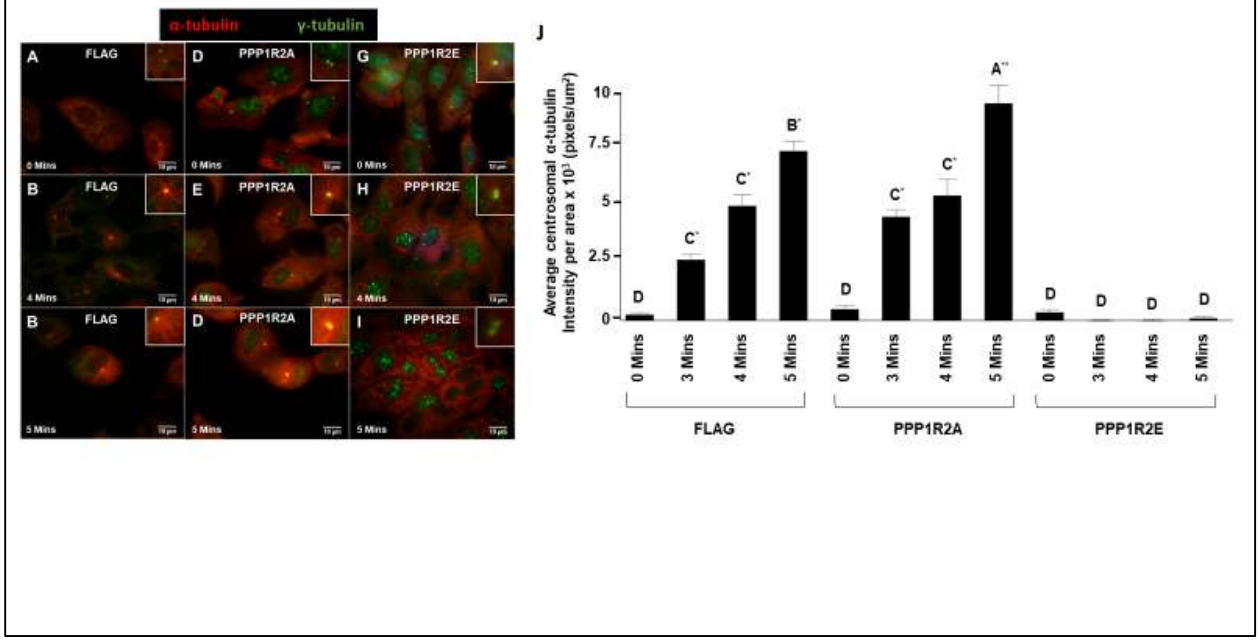
α-tubulin
γ-tubulin
A
FLAG
0 Mins
D
PPP1R2A
0 Mins
G
PPP1R2E
0 Mins
B
FLAG
4 Mins
E
PPP1R2A
4 Mins
H
PPP1R2E
4 Mins
B
FLAG
5 Mins
D
PPP1R2A
5 Mins
I
PPP1R2E
5 Mins
J
Average centrosomal α-tubulin Intensity per area x 10³ (pixels/um²)
10
7.5
5
2.5
0
D
C`
C`
B`
D
C`
C`
A``
D
D
D
D
0 Mins
3 Mins
4 Mins
5 Mins
0 Mins
3 Mins
4 Mins
5 Mins
0 Mins
3 Mins
4 Mins
5 Mins
FLAG
PPP1R2A
PPP1R2E

Figure 3.5. PPP1R2 phosphorylation regulates PPP1R2 suppression of microtubule nucleation. (A-I) ARPE-19 cells were treated with nocodazole to depolymerize microtubules. Transfected cells were allowed to recover nucleate microtubules from the centrosome after nocodazole washout over the indicated time course. Transfected cells were stained for γ-tubulin (green) and α-tubulin (red). (J) α-tubulin centrosome localization was quantified in a minimum of 100 cells for each treatment group in three replicates. Size bars equal 10 μm. Graphical representation of α-tubulin centrosome localization in cells transfected with each of the indicated plasmids. Statistically significant differences ($p<0.05$) between groups are indicated by differing letter notations above the bars and error bars represent standard error of the mean. Statistically significant differences are indicated with asterisks (*=$p\leq0.01$, **=$p\leq0.001$) compared to control.

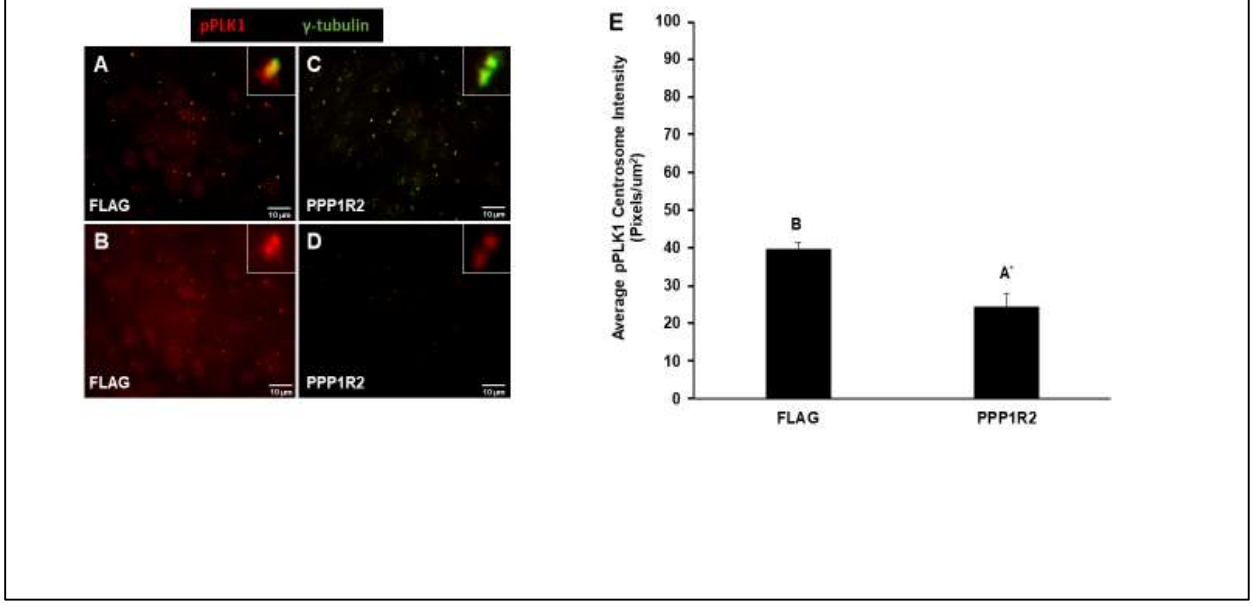
pPLK1
γ-tubulin
A
C
FLAG
PPP1R2
B
D
FLAG
PPP1R2
E
Average pPLK1 Centrosome Intensity (Pixels/um²)
100
90
80
70
60
50
40
30
20
10
0
B
A`
FLAG
PPP1R2

Figure 3.6. PPP1R2 regulates pPlk1 levels at the centrosome. (A-D) Transfected cells were stained for γ-tubulin (green) and pPlk1 (red). (B-D) Panels depicting pPlk1 (red) localization channel only. (E) α-tubulin centrosome localization was quantified in a minimum of 100 cells for each treatment group in three replicates. Size bars equal 10 μm. (E) Graphical representation of pPlk1 centrosome localization

E. Discussion

Here I investigated PPP1R2's regulation of centrosome protein recruitment and microtubule nucleation. I found that PPP1R2 interacts with both AURKA and PP1 to maintain γ-tubulin centrosome recruitment and PPP1R2 negatively regulates centrosome microtubule nucleation. PPP1R2 regulation of centrosome protein recruitment and microtubule nucleation is dependent on its phosphorylation state. Finally, PPP1R2 regulates phosphorylation levels of Plk1 at the centrosome in addition to its regulation of AURKA and PP1 phosphorylation levels as previously reported (Bresch, A.M.B., Yerich, N., Wang, R., Sperry, A. O., 2020).

AURKA, Plk1, and PP1 each regulate the centrosome at several overlapping points during the centrosome cycle (Carmena, M., & Earnshaw, W. C., 2003; Joukov, V., et al., 2014; Joukov, V., & De Nicolo, A., 2018; Kim, J., Lee, K., & Rhee, K., 2015; Lee, K., & Rhee, K., 2011; Lukasiewicz, K. B., 2009; Magnaghi-Jaulin, L., Eot-Houllier, G., Gallaud, E., & Giet, R., 2019; Mi, J., Guo, C., Brautigan, D. L. & Larner, J. M., 2007; Nasa., I. et al., 2017). AURKA, Plk1, and PP1 complex at the centrosome during centrosome maturation, but it remains unclear how PP1 interacts with AURKA and Plk1 despite all three forming a complex with Cep192 (Joukov, V. et al., 2014; Nasa, I. et al., 2017). This work addresses the incomplete understanding of centrosome maturation by describing a role for PPP1R2 in regulating PP1, AURKA, and Plk1 phosphorylation at the centrosome that is consistent with the data presented in Chapter II. Overexpression of PPP1R2, AURKA, and PP1 disrupted PCM integrity (Figure 3.1). This is consistent with the localization of AURKA and PP1 at the centrosome and their role in regulation of centrosome maturation (Joukov, V. et al., 2014; Nasa I., 2017). In addition, PPP1R2 cooverexpression with AURKA restored γ-tubulin centrosome recruitment while PPP1R2 cooverexpression with PP1 resulted in an increase in mislocalized γ-tubulin in the cytoplasm (Figure 3.1E,G-H). A similar functional interaction was seen in Chapter II where PPP1R2 cooverexpression with AURKA resulted in recovery of centrosome number and

PPP1R2 cooverexpression with PP1 and exacerbated abnormal centrosome number. This suggests that PPP1R2 has a role in coordinating AURKA and PP1 activities to regulate centrosome protein recruitment.

Results described in Chapter II demonstrated that PPP1R2 regulates the activity of both PP1 and AURKA to modulate centrosome number, midbody architecture and AURKA as well as PP1 phosphorylation at the centrosome (Bresch, A.M.B., Yerich, N., Wang, R., Sperry, A. O., 2020). Here, I discovered that PPP1R2 overexpression induced γ-tubulin mislocalization. To further investigate how PPP1R2 affects centrosome protein recruitment I co-overexpressed PPP1R2 with AURKA and PP1. PPP1R2 cooverexpression with AURKA and PP1 altered γ-tubulin localization compared to PPP1R2 alone, suggesting that PPP1R2 regulates PCM stability through both AURKA and PP1. I next used PPP1R2 truncation mutants to disrupt PPP1R2's association with AURKA and PP1 by eliminating PPP1R2 residues that discreetly interact with each enzyme. Overexpression of both PPP1R2 truncation mutants resulted in increased γ-tubulin mislocalization compared to controls. This demonstrates that PPP1R2 interacts with both PP1 and AURKA to regulate centrosome protein recruitment. Altogether, this suggests a newly-described role for PPP1R2 in PCM integrity maintenance. A similar regulatory pathway was previously established where PPP1R2 regulated the midbody through modulation of AURKA and PP1 activity resulting in proper centrosome number through cytokinesis regulation (Bresch, A.M.B., Yerich, N., Wang, R., Sperry, A. O., 2020). In Chapter II, PPP1R2 positively regulated AURKA and negatively regulated PP1 to affect midbody function. It remains unclear whether PPP1R2 has a similar effect on AURKA and PP1 activity during centrosome maturation and protein recruitment. Further experiments will be needed to assess whether PPP1R2 complexed with Cep192 inactivates AURKA and activates PP1 at the centrosome. Altogether this work expands PPP1R2's coordinate role in regulating proper midbody architecture to regulation of centrosome maturation. PPP1R2's novel role in centrosome maturation corroborates previous reports demonstrating two PPP1R2 effectors, AURKA and PP1, regulate centrosome maturation (Joukov, V., & De Nicolo, A., 2014; Nasa, I. et al., 2021). In addition, PPP1R2's coordination of enzyme activity

has also been reported at the midbody where it balances both PP1 and AURKB activity (Wang, W., Stukenberg, P. T., Brautigan, D. L., 2008).

PPP1R2's inhibition of PP1 is dependent upon PPP1R2's phosphorylation at Thr72 (Li, M., et al., 2007). Overexpression of phospho-mutants at Thr72 restored γ-tubulin centrosome recruitment (Figure 3.2E-F,G). This suggests that PPP1R2 phosphorylation at Thr72 is responsible for PPP1R2's regulation of γ-tubulin localization at the centrosome. Further experiments will be necessary to determine how overexpression of PPP1R2 phosphomutants affects the activity level of AURKA, PP1, and Plk1 as well as levels of phospho-AURKA, phospho-PP1, and phospho-Plk1 at the centrosome. This finding differs from those presented in previous reports where overexpression of PPP1R2 phosphomimetic resulted in supernumerary centrosomes and the phospho-null restored centrosome number to control levels (Bresch, A.M.B., Yerich, N., Wang, R., Sperry, A. O., 2020). Altogether, these data suggest that PPP1R2 phosphorylation regulates centrosome protein recruitment differently than centrosome number. Further investigation into the effect of phospho-null as well as phospho-mimetic overexpression on PP1, AURKA, and Plk1 activity at the centrosome will be needed to determine why both phospho-null and phospho-mimetic mutant overexpression resulted in restoration of γ-tubulin centrosome recruitment. Assessing PPP1R2 phospho-mutant overexpression's effect on the activity of PP1, AURKA, and Plk1 will clarify how PPP1R2 phosphorylation alters enzyme activity to restore proper protein recruitment to the centrosome. This will further expand on previous reports showing PPP1R2 balancing enzyme activity to regulate centrosome and midbody function (Bresch, A.M.B., Yerich, N., Wang, R., Sperry, A. O., 2020; Joukov, V., & De Nicolo, A., 2014; Nasa, I. et al., 2021; Wang, W., Stukenberg, P. T., Brautigan, D. L., 2008).

F. Conclusion

I conclude that PPP1R2 has a critical role in regulating protein recruitment at the centrosome through modulating the phosphorylation state of centrosome enzymes AURKA, PP1, and Plk1. This is

supported by previous reports which show PPP1R2 overexpression decreasing AURKA activity and increasing PP1 activity resulting in reduced phosphorylation levels of AURKA and PP1 at the centrosome (Bresch, A.M.B., Yerich, N., Wang, R., Sperry, A. O., 2020). This is corroborated by my findings where PPP1R2 overexpression also reduced Plk1 phosphorylation levels at the centrosome. The results of this study also demonstrate that PPP1R2 regulates PCM stability and microtubule nucleation by modulating enzyme phosphorylation levels at the centrosome. This is consistent with PPP1R2 overexpression reducing AURKA, PP1, and Plk1 phosphorylation at the centrosome (Bresch, A.M.B., Yerich, N., Wang, R., Sperry, A. O., 2020). This conclusion is also consistent with the increase in γ-tubulin and pericentrin cytoplasmic localization after PPP1R2 overexpression. Furthermore, I conclude that PPP1R2 has a role in microtubule nucleation regulation. This is supported by PPP1R2 and PPP1R2 phosphomimetic overexpression's inhibition of microtubule nucleation, likely through PCM instability. These data strongly support a role for PPP1R2 in regulating centrosome maturation. I propose a model where PPP1R2 suppresses AURKA activity to inactivate PP1 and Plk1 resulting in decreased protein localization at the centrosome causing suppression of microtubule nucleation. Future experiments will be needed to establish PPP1R2 as a regulator of centrosome maturation and to further define its effect on Plk1 activity as well as centrosome scaffold protein phosphorylation.

CHAPTER IV: SUMMARY

This dissertation sought to address several gaps in knowledge regarding PPP1R2's role in centrosome biology and its interactions with AURKA and PP1. PPP1R2 has been shown to regulate AURKA activity, however these studies were conducted *in vitro* with recombinant proteins. PPP1R2 is a known regulator of PP1 during centrosome separation (Eto, M., Elliott, E., Prickett, T. D., & Brautigan, D. L., 2002; Satinover, D. L., Leach, C. A., Stukenberg, P. T., & Brautigan, D. L., 2004). AURKA and PP1 are critical regulators of the centrosome throughout the centrosome cycle including centrosome maturation and centrosome separation, (Mi, J., Guo, C., Brautigan, D. L., & Larner, J. M., 2007; Joukov, V., Walter, J. C., & De Nicolo, A., 2014; Mi, J., Nasa, I., Trinkle-Mulcahy, L., Douglas, P., Chaudhuri, S., Lees-Miller, S. P., Lee, K. S., et al. 2017) however, it is unknown how PPP1R2 regulates centrosome maturation (Eto, M., Elliott, E., Prickett, T. D., & Brautigan, D. L., 2002; Helps, N. R., Luo, X., Barker, H. M., & Cohen, P. T., 2000).

This work tested the central hypothesis that PPP1R2 is a key regulator of the centrosome cycle through its interaction with AURKA and PP1. To test this, I overexpressed PPP1R2, PPP1R2 mutants, AURKA, and PP1 and then assessed centrosome and midbody structure and function by measuring protein localization at the centrosome and midbody, enzyme activity, and phosphorylation levels of centrosome related enzymes. Overall, I investigated PPP1R2's interaction with AURKA to determine how PPP1R2 modulates AURKA and PP1 activity to affect centrosome function (Joukov, V., Walter, J. C., & De Nicolo, A., 2014; Joukov, V., & De Nicolo, A., 2018a; Li, Satinover, D. L., & Brautigan, D. L., 2007; Meraldi, P., Honda, R., & Nigg, E. A., 2002; Peel N., et al., 2017; Satinover, D. L., Leach, C. A., Stukenberg, P. T., & Brautigan, D. L., 2004). I found that PPP1R2 overexpression resulted in increased centrosome number, pericentrin mislocalization, and γ-tubulin mislocalization. AURKA and PP1 overexpression had statistically similar effects on centrosome number and PCM protein localization. In addition, PPP1R2 overexpression resulted in suppression of microtubule nucleation. I propose that PPP1R2 has a role in

regulating protein recruitment at the centrosome which affects both PCM integrity and microtubule nucleation.

There are pitfalls regarding my overexpression model that challenge the proposed role of PPP1R2 overexpression specifically affecting centrosome structure and function. These pitfalls include the global overexpression of PPP1R2, PPP1R2 mutants, AURKA, and PP1 in asynchronous cells. Effects of this global overexpression can result in deregulation of phosphorylation outside of the centrosome and throughout the cell cycle. Cells were able to enter mitosis and undergo cell division despite having multiple centrosome defects suggesting that cell cycle checkpoints were not activated by the abnormalities. Cell cycle checkpoints are driven by enzyme activity tightly regulating phosphorylation levels and involve cyclin dependent kinases (CDKs). It is possible that this overexpression model perturbed phosphorylation levels outside the centrosome and midbody resulting in uncontrolled cell cycle progression despite the establishment of centrosome amplification and abnormal chromosome number. There are other enzymes besides AURKA and PP1 that were likely affected throughout the cell cycle by this overexpression model. If this is the case, then deregulation of enzyme activity outside of AURKA and PP1 could also contribute to the outcome of these experiments.

I unexpectedly discovered a novel role for PPP1R2 in regulating midbody architecture while testing the central hypothesis. A previous report determined that PPP1R2 balances PP1 and AURKB activity during cytokinesis (Wang, W., Stukenberg, P. T., & Brautigan, D. L., 2008). My discovery and the previous report led me to formulate a new hypothesis, that PPP1R2 regulates PP1 and AURKA activity to maintain midbody structure and function to ensure effective cytokinesis. Testing this hypothesis, I determined that PPP1R2 regulates PP1 midbody recruitment. Results suggested the increase in centrosome frequency after PPP1R2 overexpression was due indirectly to a cytokinesis defect. These results are consistent with a new role for PPP1R2 in regulating both cytokinesis and centrosome protein recruitment.

PPP1R2 overexpression increased both midbody length and frequency of abnormal midbody morphology. Overexpression of PPP1R2 truncation mutants significantly decreased PP1 midbody recruitment and PPP1R2 overexpression significantly increased PP1 midbody recruitment. Together, these data suggest that PPP1R2 regulates PP1 midbody recruitment maintain midbody morphology. This is consistent with previous reports that demonstrate that PPP1R2 regulates PP1 activity at the midbody (Wang, W., Stukenberg, P. T., & Brautigan, D. L., 2008). I also demonstrated that PPP1R2's regulation of cytokinesis indirectly maintains proper centrosome number. I propose that PPP1R2 recruits PP1 to the midbody and regulates its activity there to maintain proper midbody architecture and function. Further investigation will be necessary to determine how PPP1R2 recruits PP1 to the midbody and how this affects midbody architecture and centrosome number.

This work demonstrated that PPP1R2 overexpression increased PP1 activity, decreased AURKA activity, and reduced PP1, AURKA, and Plk1 phosphorylation at the centrosome. PPP1R2 overexpression inactivates AURKA to increase PP1 activity and disrupt centrosome function. These data describe a novel mechanism whereby PPP1R2 inactivates AURKA to indirectly activate PP1. The finding that PPP1R2 affects both AURKA and PP1 is contrary to previous reports that PPP1R2 affects downstream enzyme activity through PP1 activity modulation alone (Eto, M. et al., 2002; Wang, W., Stukenberg, P. T., & Brautigan, D. L., 2008). However, this study determined that PPP1R2 can modulate both PP1 and AURKA activities. My data establish PPP1R2 as a coordinator of both PP1 and AURKA activity. This mechanism includes PPP1R2 regulation of enzyme activity through inactivation of AURKA to activate PP1 and challenges the previously established mechanism where PPP1R2 inactivated PP1 alone to regulate downstream enzyme activity (Eto, M.et al., 2002; Wang, W. et al., 2008).

PPP1R2 has been shown to have the ability to interact with AURKA and PP1 through discreet residues, but it remains unknown whether PPP1R2 forms strictly dimeric complexes or trimeric complexes with AURKA and PP1. A previous report stated that PPP1R2 can form trimeric complexes with PP1 and

AURKA as well as TPX2 and AURKA (Satinover, D. L. et al., 2004), but it remains unknown whether PPP1R2 could directly regulate multiple enzymes within a complex. This work demonstrates that PPP1R2 overexpression can alter the activity of both PP1 and AURKA at the centrosome. This is consistent with PPP1R2 directly interacting with both PP1 and AURKA. I propose that PPP1R2 has a more complex relationship with its associated enzymes than previously thought. Further investigation will be necessary to assess how PPP1R2 regulates enzyme activity of other protein complexes including the NEK2 and AURKB pathways (Eto, M.et al., 2002; Wang, W. et al., 2008).

This work raises three outstanding questions. *First, does PPP1R2 regulate the phosphorylation of critical PCM proteins Cep192 and pericentrin through inactivation of AURKA and Plk1?* PPP1R2 overexpression decreased phosphorylation of centrosome enzymes including AURKA, PP1, and Plk1 (Bresch, A.M.B., Yerich, N., Wang, R., Sperry, A. O., 2020). Since PPP1R2 overexpression significantly decreased phosphorylation of centrosome enzymes, it is likely that PPP1R2 overexpression will also decrease phosphorylation levels of their downstream substrates, including Cep192 and pericentrin. Reduced Cep192 and pericentrin phosphorylation is consistent with the effect of PPP1R2 on disruption of γ-tubulin and pericentrin recruitment to the centrosome. This is supported by previous reports that show AURKA phosphorylates Plk to begin a phosphorylation cascade necessary for microtubule nucleation at the centrosome. Plk1 also phosphorylates pericentrin to initiate centrosome maturation (Lee, K. & Rhee, 2011). Therefore, I predict that PPP1R2 overexpression will disrupt this signaling cascade to lower phosphorylation of Cep192 and pericentrin, which will disrupt γ-tubulin and pericentrin recruitment to the centrosome.

Second, does inactivation of AURKA when PPP1R2 is overexpressed allow affected cells to bypass the G_2/M checkpoint? The G_2/M cell cycle checkpoint immediately follows centrosome maturation (Meraldi, P., & Nigg, E. A. 2002). PPP1R2 overexpression induced an increase in multipolar spindle formation concurrent with increased centrosome number (Figure 4.1). PPP1R2 overexpression also induced

supernumerary centrosomes through inactivation of AURKA (Figure 2.8). Therefore, PPP1R2 may regulate the G_2/M checkpoint through modulation of AURKA activity. Investigating how PPP1R2 overexpression enables cells with supernumerary centrosomes to bypass cell cycle checkpoints, specifically the G_2/M phase transition, will be critical to understanding how PPP1R2 overexpression drives multipolar spindle formation. It is already known that AURKA plays a critical role in regulating both the G_2/M transition (Asteriti, I. A., De Mattia, F., & Guarguaglini, G., 2015; Joukov, V., & De Nicolo, A., 2018). AURKA regulates G_2/M transition through direct interaction with Bora, and Plk1 (Asteriti, I. A. et al., 2015). Bora is a cofactor for AURKA that increases AURKA phosphorylation of Plk1 through Plk1 conformational change. AURKA and Bora cooperatively activate Plk1 to allow transition of the cell cycle phase into mitosis (Asteriti, I. A. et al., 2015).Future experiments will test a model where PPP1R2 modulates AURKA activity in complex with Bora and Plk1 during the G_2/M transition.

Third, does PPP1R2 regulate PP1 to recruit abscission machinery to the midbody to complete cytokinesis? PP1 opposes Plk1 phosphorylation of Cep55 at Ser436, resulting in Cep55 midbody recruitment. Cep55 subsequently recruits ESCRT complexes to the midbody to induce abscission, (Gao, K. et al., 2018; Lee, H. H., Elia, N., Ghirlando, R., Lippincott-Schwartz, J., & Hurley, J. H., 2008). My findings show that removal of either PPP1R2 termini significantly reduces PP1 midbody localization (Figure 2.5). Therefore, PPP1R2 could regulate PP1 at the midbody, resulting in downstream recruitment of the Cep55 and ESCRT complex to the midbody. I conclude that PPP1R2 interacts with PP1 and related complexes at critical residues throughout its length to regulate PP1 midbody localization.

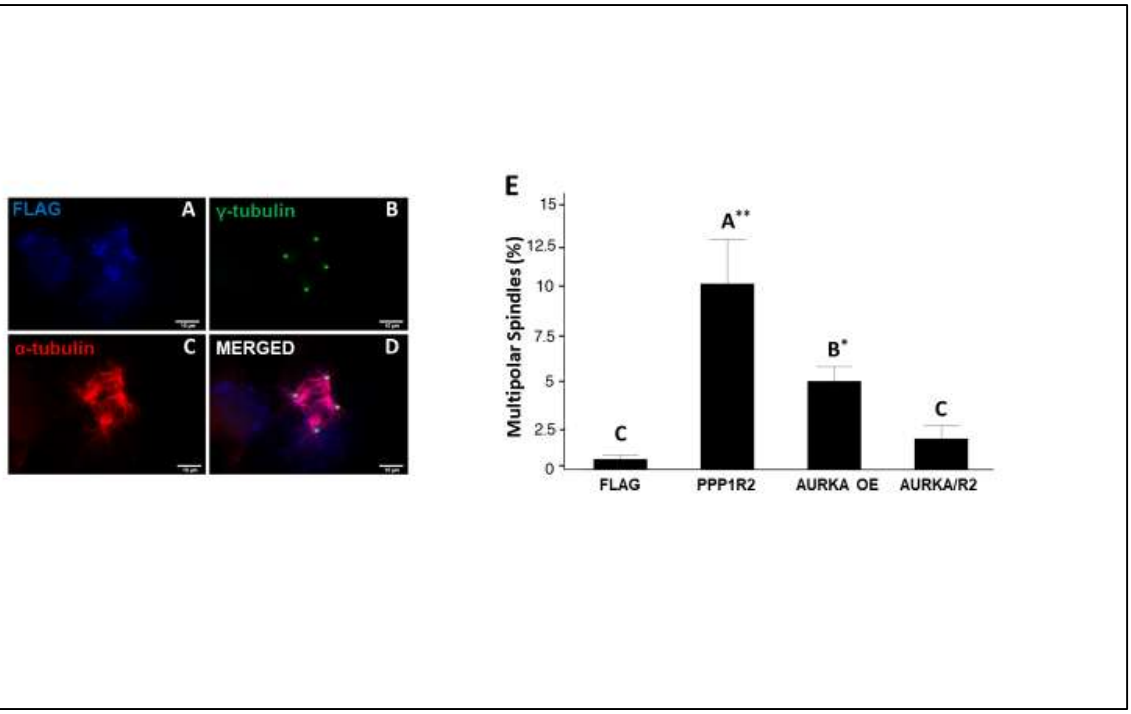
FLAG
A
γ-tubulin
B
α-tubulin
C
MERGED
D
E
Multipolar Spindles (%)
15
12.5
10
7.5
5
2.5
0
A**
B*
C
C
FLAG
PPP1R2
AURKA OE
AURKA/R2

Figure 4.1. PPP1R2 and AURKA overexpression significantly increases multipolar spindle frequency. Cells were transfected and overexpressed either PPP1R2, AURKA, PPP1R2/AURKA, or FLAG as an empty vector control. (A-D) Cells were then labeled with antibodies targeting FLAG (blue), γ-tubulin (green), or α-tubulin (red). (E) Graphical representation multipolar spindle frequency in cells transfected

Based on my results, I propose a model whereby PPP1R2 plays a role similar to that of iASPP in PP1 recruitment during cytokinesis. iASPP is a protein in the same protein phosphatase regulatory family as PPP1R2 and is also a regulatory subunit of PP1 (Gao, K. et al., 2018). iASPP is essential for PP1 midbody recruitment and is involved in facilitating PP1's interaction with Cep55 (Gao, K. et al., 2018).siRNA mediated reduction of iASPP decreased PP1 midbody localization resulting in loss of ESCRT III at the midbody (Gao, K. et al., 2018). My results determined that PPP1R2 shares a similar role to iASPP and regulates PP1 during PP1 midbody recruitment. Altogether these data support further experiments to determine which domains of PPP1R2 are involved in ESCRT complex recruitment. Future experiments will also use siRNA to knockdown PPP1R2 and assess its effect on ESCRT complex midbody localization.

This work provides strong evidence that PPP1R2 coordinates AURKA and PP1 activity to critically regulate centrosome protein recruitment and midbody architecture. I also demonstrate that PPP1R2 maintains AURKA, PP1, and PLk1 phosphorylation levels at the centrosome. I propose a novel mechanism through PPP1R2 inactivation of AURKA resulting in PP1 activation and lower Plk1 activity at the centrosome. These data demonstrate that PPP1R2 maintains centrosome protein recruitment to regulate PCM stability and microtubule nucleation. In addition, PPP1R2 indirectly impacts centrosome number through regulation of cytokinesis.

These conclusions have been drawn through an experimental model using only one cell line: retinal pigmented epithelial (ARPE) cells. Additional cell lines will need to be assessed using the same model to determine whether PPP1R2 regulates the centrosome and midbody in a conserved manner across multiple cell types. Additional cells types that are effective transfection candidates include PE cells, WI 38 fibroblast-like fetal lung cells, COS-7 cells, and HEK293 cells.

It remains unclear whether PPP1R2 overexpression's deregulation of centrosome function, midbody architecture, multipolar spindles, and resultant chromosome defects would lead to tumorigenesis. Centrosome and mitotic spindle dysfunction has been correlated to tumorigenesis, but the current model is not sufficient to address whether these would lead to abnormal cell functions that later establish cancer. Currently, the overexpression model is transient and only occurs over a 24 hour time period. A stably transfected cell line will have to be established that constitutively overexpresses PPP1R2 to study the long-term effects of PPP1R2 overexpression on the centrosome and cell division. This cell line would allow the assessment of cancer related characteristics in cell populations that have overexpressed PPP1R2 over multiple rounds of cell division. Future studies would be needed to compare cell populations that have underwent multiple passages overexpressing PPP1R2 to untreated cell populations serving as a control.

REFERENCES

Addi, C., Bai, J., and Echard, A. (2018). Actin, microtubule, septin and ESCRT filament remodeling during late steps of cytokinesis. Current Opinion in Cell Biology, 50, 27-34. doi: 10.1016/j.ceb.2018.01.007.

Adon, A. M., Zeng, X., Harrison, M. K., Sannem, S., Kiyokawa, H., Kaldis, P., and Saavedra, H. I. (2010). Cdk2 and Cdk4 regulate the centrosome cycle and are critical mediators of centrosome amplification in p53-null cells. Molecular and Cellular Biology, 30(3), 694-710. doi: 10.1128/MCB.00253-09.

Adriaans, I. E., Basant, A., Ponsioen, B., Glotzer, M., and Lens, S. M. A. (2019). Plk1 plays dual roles in centralspindlin regulation during cytokinesis. The Journal of Cell Biology, 218(4), 1250-1264. doi: 10.1083/jcb.201805036.

Antanavičiūtė, I., Gibieža, P., Prekeris, R., and Skeberdis, V. A. (2018). Midbody: From the regulator of cytokinesis to postmitotic signaling organelle. Medicina, 54(4), 53. doi: 10.3390/medicina54040053.

Archambault, V., Lépine, G., and Kachaner, D. (2015). Understanding the polo kinase machine. Oncogene, 34(37), 4799-4807. doi: 10.1038/onc.2014.451.

Arlot-Bonnemains, Y., and Prigent, C. (2002). Cell cycle. A trigger for centrosome duplication. Science, 295(5554), 455-456. doi: 10.1126/science.1068917.

Arnaoutov, A., and Dasso, M. (2005). Ran-GTP regulates kinetochore attachment in somatic cells. Cell Cycle, 4(9), 1161-1165. doi: 10.4161/cc.4.9.1979.

Asteriti, I. A., De Mattia, F., & Guarguaglini, G. (2015). Cross-talk between AURKA and Plk1 in mitotic entry and spindle assembly. Frontiers in Oncology*, 5*, 283. doi: 10.3389/fonc.2015.00283.

Azmi, I., Davies, B., Dimaano, C., Payne, J., Eckert, D., Babst, M., and Katzmann, D. J. (2006). Recycling of ESCRTs by the AAA-ATPase Vps4 is regulated by a conserved VSL region in Vta1. Journal of Cell Biology, 172(5), 705-717. doi: 10.1083/jcb.200508166.

Bakhoum, S. F., Thompson, S. L., Manning, A. L., and Compton, D. A. (2009). Genome stability is ensured by temporal control of kinetochore-microtubule dynamics. Nature Cell Biology, 11(1), 27-35. doi: 10.1038/ncb1809.

Bassi, Z. I., Audusseau, M., Riparbelli, M. G., Callaini, G., and D'Avino, P. P. (2013). Citron kinase controls a molecular network required for midbody formation in cytokinesis. Proceedings of the National Academy of Sciences of the United States of America, 110(24), 9782-9787. doi: 10.1073/pnas.1301328110.

Bastos, R. N., and Barr, F. A. (2010). Plk1 negatively regulates Cep55 recruitment to the midbody to ensure orderly abscission. The Journal of Cell Biology, 191(4), 751-760. doi: 10.1083/jcb.201008108.

Bayliss, R., Sardon, T., Ebert, J., Lindner, D., Vernos, I., and Conti, E. (2004). Determinants for Aurora-A activation and Aurora-B discrimination by TPX2. Cell Cycle, 3(4), 404-407. doi: 10.4161/cc.3.4.777

Bayliss, R., Sardon, T., Vernos, I., and Conti, E. (2003). Structural basis of Aurora-A activation by TPX2 at the mitotic spindle. Molecular Cell, 12(4), 851-862. doi: 10.1016/s1097-2765(03)00392-7.

Bertolin, G., Sizaire, F., Herbomel, G., Reboutier, D., Prigent, C., and Tramier, M. (2016). A FRET biosensor reveals spatiotemporal activation and functions of aurora kinase A in living cells. Nature Communications, 7, 12674. doi: 10.1038/ncomms12674.

Bhowmick, R., Thakur, R. S., Venegas, A. B., Liu, Y., Nilsson, J., Barisic, M., and Hickson, I. D. (2019). The RIF1-PP1 axis controls abscission timing in human cells. Current Biology, 29(7), 1232-1242.e5. doi: 10.1016/j.cub.2019.02.037.

Bieling, P., Telley, I. A., and Surrey, T. (2010). A minimal midzone protein module controls formation and length of antiparallel microtubule overlaps. Cell, 142(3), 420-432. doi: 10.1016/j.cell.2010.06.033.

Bollen M, Peti W, Ragusa MJ, Beullens M. (2010). The extended PP1 toolkit: Designed to create specificity. Trends in Biochemical Sciences, 35(8), 450-458. doi: 10.1016/j.tibs.2010.03.002.

Bowne-Anderson, H., Zanic, M., Kauer, M., and Howard, J. (2013). Microtubule dynamic instability: A new model with coupled GTP hydrolysis and multistep catastrophe. BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology, 35(5), 452-461. doi: 10.1002/bies.201200131.

Brautigan DL, Sunwoo J, Labbe JC, Fernandez A, Lamb NJ. (1990). Cell cycle oscillation of phosphatase inhibitor-2 in rat fibroblasts coincident with p34cdc2 restriction. Nature Review Molecular Cell Biology, 344(6261), 74-78. doi: 10.1038/344074a0.

Breslow, D. K., and Holland, A. J. (2019). Mechanism and regulation of centriole and cilium biogenesis. Annual Review of Biochemistry, 88, 691-724. doi: 10.1146/annurev-biochem-013118-111153.

Burgess, S. G., Peset, I., Joseph, N., Cavazza, T., Vernos, I., Pfuhl, M., Gergely, F., Bayliss, R. (2015). Aurora-A-dependent control of TACC3 influences the rate of mitotic spindle assembly. PLoS Genetics, 11(7), e1005345. doi: 10.1371/journal.pgen.1005345.

Burke, D. J., and Stukenberg, P. T. (2008). Linking kinetochore-microtubule binding to the spindle checkpoint. Developmental Cell, 14(4), 474-479. doi: 10.1016/j.devcel.2008.03.015.

Capalbo L, Bassi ZI, Geymonat M, Todesca S, Copoiu L, Enright AJ, Callaini G, Riparbelli MG, Yu L, Choudhary JS. (2019). The midbody interactome reveals unexpected roles for PP1 phosphatases in cytokinesis. Nature Communications, 10(1), 4513. doi: 10.1038/s41467-019-12507-9.

Carazo-Salas, R. E., Guarguaglini, G., Gruss, O. J., Segref, A., Karsenti, E., and Mattaj, I. W. (1999). Generation of GTP-bound Ran by RCC1 is required for chromatin-induced mitotic spindle formation. Nature, 400(6740), 178-181. doi: 10.1038/22133.

Carmena M, E. W. (2003). The cellular geography of aurora kinases. Nature Review Molecular Cell Biology, 4(11), 842-854. doi: 10.1038/nrm1245.

Ceulemans H, B. M. (2004). Functional diversity of protein phosphatase-1, a cellular economizer and reset button. Physiological Reviews, 84(1), 39. doi: 10.1152/physrev.00013.2003.

Chen, M., Cao, Y., Dong, D., Zhang, Z., Zhang, Y., Chen, J., Liu, M. (2019). Regulation of mitotic spindle orientation by phosphorylation of end binding protein 1. Experimental Cell Research, 384(1), 111618. doi: 10.1016/j.yexcr.2019.111618.

Cheng, H. W., Hsiao, C. T., Chen, Y. Q., Huang, C. M., Chan, S. I., Chiou, A., & Kuo, J. C. (2019). Centrosome guides spatial activation of rac to control cell polarization and directed cell migration. Life Science Alliance, 2(1), e201800135. doi: 10.26508/lsa.201800135. Print 2019 Feb. doi: 10.26508/lsa.201800135.

Chou, C. H., Loh, J. K., Yang, M. C., Lin, C. C., Hong, M. C., Cho, C. L., Chou, A. K., Wang, C. H., Lieu, A. S., Howng, S. L., Hsu, C. M., (2015). AIBp regulates mitotic entry and mitotic spindle assembly by controlling activation of both Aurora-A and Plk1. Cell Cycle, 14(17), 2764-2776. doi: 10.1080/15384101.2015.1066536.

Christ, L., Raiborg, C., Wenzel, E. M., Campsteijn, C., and Stenmark, H. (2017). Cellular functions and molecular mechanisms of the ESCRT membrane-scission machinery. Trends in Biochemical Sciences, 42(1), 42-56. doi: 10.1016/j.tibs.2016.08.016.

Christ, L., Wenzel, E. M., Liestøl, K., Raiborg, C., Campsteijn, C., and Stenmark, H. (2016). ALIX and ESCRT-I/II function as parallel ESCRT-III recruiters in cytokinetic abscission. The Journal of Cell Biology, 212(5), 499-513. doi: 10.1083/jcb.201507009.

Cohen P. (1989). The structure and regulation of protein phosphatases. Annual Review of Biochemistry, 58, 453-508. doi: 10.1146/annurev.bi.58.070189.00232.

Colicino, E. G., and Hehnly, H. (2018). Regulating a key mitotic regulator, polo-like kinase 1 (PLK1). Cytoskeleton, 75(11), 481-494. doi: 10.1002/cm.21504.

Combes, G., Alharbi, I., Braga, L. G., and Elowe, S. (2017). Playing polo during mitosis: PLK1 takes the lead. Oncogene, 36(34), 4819-4827. doi: 10.1038/onc.2017.113.

Conduit, P., T., Wainman, A., and Raff, J.W., (2015). Centrosome function and assembly in animal cells. Nature Reviews Molecular Cell Biology, 16, 611–624. doi: 10.1038/nrm4062

Conduit, P. T., Brunk, K., Dobbelaere, J., Dix, C. I., Lucas, E. P., and Raff, J. W. (2010). Centrioles regulate centrosome size by controlling the rate of Cnn incorporation into the PCM. Current Biology, 20(24), 2178-2186. doi: 10.1016/j.cub.2010.11.011.

Conduit, P. T., Feng, Z., Richens, J. H., Baumbach, J., Wainman, A., Bakshi, S. D., Raff, J. W. (2014). The centrosome-specific phosphorylation of Cnn by Polo/Plk1 drives Cnn scaffold assembly and centrosome maturation. Developmental Cell, 28(6), 659-669. doi: 10.1016/j.devcel.2014.02.013.

Connor, J. H., Frederick, D., Huang, H., Yang, J., Helps, N. R., Cohen, P. T., Nairn, A. C., Tatchell, A. D. R. K., Shenolikar, S., (2000). Cellular mechanisms regulating protein phosphatase-1. A key functional interaction between inhibitor-2 and the type 1 protein phosphatase catalytic subunit. The Journal of Biological Chemistry, 275(25), 18670-18675.

Coombes, C. E., Yamamoto, A., Kenzie, M. R., Odde, D. J., and Gardner, M. K. (2013). Evolving tip structures can explain age-dependent microtubule catastrophe. Current Biology, 23(14), 1342-1348. doi: 10.1016/j.cub.2013.05.059.

Courthéoux, T., Reboutier, D., Vazeille, T., Cremet, J. Y., Benaud, C., Vernos, I., and Prigent, C. (2019). Microtubule nucleation during central spindle assembly requires NEDD1 phosphorylation on serine 405 by Aurora A. Journal of Cell Science, 132(10), jcs231118. doi: 10.1242/jcs.231118.

Cowley, D. O., Rivera-Pérez, J. A., Schliekelman, M., He, Y. J., Oliver, T. G., Lu, L., Van Dyke, T. (2009). Aurora-A kinase is essential for bipolar spindle formation and early development. Molecular and Cellular Biology, 29(4), 1059-1071. doi: 10.1128/MCB.01062-08.

D'Avino, P. P., and Capalbo, L. (2016). Regulation of midbody formation and function by mitotic kinases. Seminars in Cell and Developmental Biology, 53, 57-63. doi: 10.1016/j.semcdb.2016.01.018.

D'Avino, P. P., Savoian, M. S., and Glover, D. M. (2005). Cleavage furrow formation and ingression during animal cytokinesis: A microtubule legacy. Journal of Cell Science, 118(Pt 8), 1549-1558.

doi: 10.1242/jcs.02335

Dammermann, A., and Merdes, A. (2002). Assembly of centrosomal proteins and microtubule organization depends on PCM-1. The Journal of Cell Biology, 159(2), 255-266. doi: 10.1083/jcb.200204023.

Delaval, B., and Doxsey, S. J. (2010). Pericentrin in cellular function and disease. The Journal of Cell Biology, 188(2), 181-190. doi: 10.1083/jcb.200908114.

Delgehyr, N., and Spassky, N. (2014). Interplay between primary cilia and cell cycle. [Cil primaire, cycle cellulaire et prolifération] Medecine Sciences, 30(11), 976-979. doi: 10.1051/medsci/20143011011.

Desai, A., and Mitchison, T. J. (1997). Microtubule polymerization dynamics. Annual Review of Cell and Developmental Biology, 13, 83-117. doi: 10.1146/annurev.cellbio.13.1.83.

DeVaul, N., Wang, R., and Sperry, A. O. (2013). PPP1R42, a PP1 binding protein, regulates centrosome dynamics in ARPE-19 cells. Biology of the Cell, 105(8), 359-371. doi: 10.1111/boc.201300019.

Dictenberg, J. B., Zimmerman, W., Sparks, C. A., Young, A., Vidair, C., Zheng, Y., Carrington, W., Fay, F. S., Doxsey, S. J. (1998). Pericentrin and gamma-tubulin form a protein complex and are organized into a novel lattice at the centrosome. The Journal of Cell Biology, 141(1), 163-174. doi: 10.1083/jcb.141.1.163.

El-Amine, N., Carim, S. C., Wernike, D., and Hickson, G. R. X. (2019). Rho-dependent control of the citron kinase, sticky, drives midbody ring maturation. Molecular Biology of the Cell, 30(17), 2185-2204. doi: 10.1091/mbc.E19-04-0194.

Eto M, Elliott E, Prickett TD, Brautigan DL. (2002). Inhibitor-2 regulates protein phosphatase-1 complexed with NimA-related kinase to induce centrosome separation. Journal of Biological Chemistry, 277(46), 44013-44020. doi: 10.1074/jbc.M208035200.

Fabbro, M., Zhou, B. B., Takahashi, M., Sarcevic, B., Lal, P., Graham, M. E., Khanna, K. K. (2005). Cdk1/Erk2- and Plk1-dependent phosphorylation of a centrosome protein, Cep55, is required for its recruitment to midbody and cytokinesis. Developmental Cell, 9(4), 477-488. doi: 10.1016/j.devcel.2005.09.003.

Farache, D., Emorine, L., Haren, L., and Merdes, A. (2018). Assembly and regulation of gamma-tubulin complexes. Open Biology, 8(3), 170266. doi: 10.1098/rsob.170266.

Fu, W., Chen, H., Wang, G., Luo, J., Deng, Z., Xin, G., Zhang, C. (2013). Self-assembly and sorting of acentrosomal microtubules by TACC3 facilitate kinetochore capture during the mitotic spindle assembly. Proceedings of the National Academy of Sciences of the United States of America, 110(38), 15295-15300. doi: 10.1073/pnas.1312382110.

Fujita, H. Yoshino, Y. Chiba, N. (2016). Regulation of the centrosome cycle. Molecular Cell Oncology, 3(2), 1-7. doi: 10.1080/23723556.2015.1075643.

Fuller, B. G., Lampson, M. A., Foley, E. A., Rosasco-Nitcher, S., Le, K. V., Tobelmann, P., Kapoor, T. M. (2008). Midzone activation of aurora B in anaphase produces an intracellular phosphorylation gradient. Nature, 453(7198), 1132-1136. doi: 10.1038/nature06923.

Gao K, Zhang Y, Shi Q, Zhang J, Zhang L, Sun H, Jiao D, Zhao X, Tao H, Wei Y. (2018). iASPP-PP1 complex is required for cytokinetic abscission by controlling CEP55 dephosphorylation. 9(5), 528. doi: 10.1038/s41419-018-0561-6.

Gardner, M. K., Charlebois, B. D., Jánosi, I. M., Howard, J., Hunt, A. J., and Odde, D. J. (2011). Rapid microtubule self-assembly kinetics. Cell, 146(4), 582-592. doi: 10.1016/j.cell.2011.06.053.

Gemble, S., Simon, A., Pennetier, C., Dumont, M., Hervé, S., Meitinger, F., Basto, R. (2019). Centromere dysfunction compromises mitotic spindle pole integrity. Current Biology, 29(18), 3072-3080.e5. doi: 10.1016/j.cub.2019.07.052.

Giet R, McLean D, Descamps S, Lee MJ, Raff JW, Prigent C, Glover DM. (2002). Drosophila Aurora A kinase is required to localize D-TACC to centrosomes and to regulate astral microtubules. Journal of Cell Biology, 156(3), 437-451. doi: 10.1083/jcb.200108135.

Gireesh, K. K., Shine, A., Lakshmi, R. B., Vijayan, V., and Manna, T. K. (2018). GTP-binding facilitates EB1 recruitment onto microtubules by relieving its auto-inhibition. Scientific Reports, 8(1), 9792-018-28056-y. doi: 10.1038/s41598-018-28056-y.

Glover, D. M., Leibowitz, M. H., McLean, D. A., and Parry, H. (1995). Mutations in aurora prevent centrosome separation leading to the formation of monopolar spindles. Cell, 81(1), 95-105. doi: 10.1016/0092-8674(95)90374-7.

Godinho, S. A., and Pellman, D. (2014). Causes and consequences of centrosome abnormalities in cancer. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 369(1650), 20130467. doi: 10.1098/rstb.2013.0467.

Gomez-Ferreria, M. A., Bashkurov, M., Helbig, A. O., Larsen, B., Pawson, T., Gingras, A. C., and Pelletier, L. (2012). Novel NEDD1 phosphorylation sites regulate γ-tubulin binding and mitotic spindle assembly. Journal of Cell Science, 125(Pt 16), 3745-3751. doi: 10.1242/jcs.105130.

Gomez-Ferreria, M. A., Rath, U., Buster, D. W., Chanda, S. K., Caldwell, J. S., Rines, D. R., and Sharp, D. J. (2007). Human Cep192 is required for mitotic centrosome and spindle assembly. Current Biology, 17(22), 1960-1966. doi: 10.1016/j.cub.2007.10.019.

Gomez-Ferreria, M. A., and Sharp, D. J. (2008). Cep192 and the generation of the mitotic spindle. Cell Cycle, 7(11), 1507-1510. doi: 10.4161/cc.7.11.5957.

Goodson, H. V., and Jonasson, E. M. (2018). Microtubules and microtubule-associated proteins. Cold Spring Harbor Perspectives in Biology, 10(6), a022608. doi: 10.1101/cshperspect.a022608.

Gopalakrishnan, J., Mennella, V., Blachon, S., Zhai, B., Smith, A. H., Megraw, T. L., Nicastro, D., Gygi, S. P., Agard, D. A., Avidor-Reiss, T. (2011). Sas-4 provides a scaffold for cytoplasmic complexes and tethers them in a centrosome. Nature Communications, 2, 359. doi: 10.1038/ncomms1367.

Goswami, S., Korrodi-Gregório, L., Sinha, N., Bhutada, S., Bhattacharjee, R., Kline, D., & Vijayaraghavan, S. (2019). Regulators of the protein phosphatase PP1γ2, PPP1R2, PPP1R7, and PPP1R11 are involved in epididymal sperm maturation. Journal of Cellular Physiology, 234(3), 3105-3118. doi: 10.1002/jcp.27130.

Green, R. A., Mayers, J. R., Wang, S., Lewellyn, L., Desai, A., Audhya, A., and Oegema, K. (2013). The midbody ring scaffolds the abscission machinery in the absence of midbody microtubules. The Journal of Cell Biology, 203(3), 505-520. doi: 10.1083/jcb.201306036.

Greenan, G., Brangwynne, C. P., Jaensch, S., Gharakhani, J., Jülicher, F., and Hyman, A. A. (2010). Centrosome size sets mitotic spindle length in Caenorhabditis elegans embryos. Current Biology, 20(4), 353-358. doi: 10.1016/j.cub.2009.12.050.

Grigoriev, I., Borisy, G., and Vorobjev, I. (2006). Regulation of microtubule dynamics in 3T3 fibroblasts by Rho family GTPases. Cell Motility and the Cytoskeleton, 63(1), 29-40. doi: 10.1002/cm.20107.

Grishchuk, E. L., Molodtsov, M. I., Ataullakhanov, F. I., and McIntosh, J. R. (2005). Force production by disassembling microtubules. Nature, 438(7066), 384-388. doi: 10.1038/nature04132.

Guizetti, J., and Gerlich, D. W. (2010). Cytokinetic abscission in animal cells. Seminars in Cell and Developmental Biology, 21(9), 909-916. doi: 10.1038/nature04132.

Gulluni, F., Martini, M., and Hirsch, E. (2017). Cytokinetic abscission: Phosphoinositides and ESCRTs direct the final cut. Journal of Cellular Biochemistry, 118(11), 3561-3568. doi: 10.1002/jcb.26066.

Guo, L., Mohd, K. S., Ren, H., Xin, G., Jiang, Q., Clarke, P. R., and Zhang, C. (2019). Phosphorylation of importin-α1 by CDK1-cyclin B1 controls mitotic spindle assembly. Journal of Cell Science, 132(18). doi: 10.1002/jcb.26066.

Hannabuss, J., Lera-Ramirez, M., Cade, N. I., Fourniol, F. J., Nédélec, F., and Surrey, T. (2019). Self-organization of minimal anaphase spindle midzone bundles. Current Biology, 29(13), 2120-2130. doi: 10.1016/j.cub.2019.05.049.

Haren, L., Remy, M. H., Bazin, I., Callebaut, I., Wright, M., and Merdes, A. (2006). NEDD1-dependent recruitment of the gamma-tubulin ring complex to the centrosome is necessary for centriole duplication and spindle assembly. The Journal of Cell Biology, 172(4), 505-515. doi: 10.1083/jcb.200510028.

Haren, L., Stearns, T., & Lüders, J. (2009). Plk1-dependent recruitment of gamma-tubulin complexes to mitotic centrosomes involves multiple PCM components. PloS One, 4(6), e5976. doi: 10.1371/journal.pone.0005976.

Helps, N. R., Luo, X., Barker, H. M., and Cohen, P. T. (2000). NIMA-related kinase 2 (Nek2), a cell-cycle-regulated protein kinase localized to centrosomes, is complexed to protein phosphatase 1. The Biochemical Journal, 349(Pt 2), 509-518. doi: 10.1042/0264-6021:3490509.

Henne, W. M., Buchkovich, N. J., and Emr, S. D. (2011). The ESCRT pathway. Developmental Cell, 21(1), 77-91. doi: 10.1016/j.devcel.2011.05.015.

Hinchcliffe, E. H. (2014). Centrosomes and the art of mitotic spindle maintenance. International Review of Cell and Molecular Biology, 313, 179-217. doi: 10.1016/B978-0-12-800177-6.00006-2.

Hoffmann, I. (2020). Centrosomes in mitotic spindle assembly and orientation. Current Opinion in Structural Biology, 66, 193-198. doi: 10.1016/j.sbi.2020.11.003.

Holmes, C. F., Campbell, D. G., Caudwell, F. B., Aitken, A., and Cohen, P. (1986). The protein phosphatases involved in cellular regulation. primary structure of inhibitor-2 from rabbit skeletal muscle. European Journal of Biochemistry, 155(1), 173-182. doi: 10.1111/j.1432-1033.1986.tb09473.x.

Horio, T., and Murata, T. (2014). The role of dynamic instability in microtubule organization. Frontiers in Plant Science, 5, 511. doi: 10.3389/fpls.2014.00511.

Howard, J., and Hyman, A. A. (2003). Dynamics and mechanics of the microtubule plus end. Nature, 422(6933), 753-758. doi: 10.1038/nature01600.

Hu, C. K., Coughlin, M., and Mitchison, T. J. (2012). Midbody assembly and its regulation during cytokinesis. Molecular Biology of the Cell, 23(6), 1024-1034. doi: 10.1091/mbc.E11-08-0721.

Hurley, J. H., and Emr, S. D. (2006). The ESCRT complexes: Structure and mechanism of a membrane-trafficking network. Annual Review of Biophysics and Biomolecular Structure, 35, 277-298. doi: 10.1146/annurev.biophys.35.040405.102126.

Hyman, A. A., Chrétien, D., Arnal, I., and Wade, R. H. (1995). Structural changes accompanying GTP hydrolysis in microtubules: Information from a slowly hydrolyzable analogue guanylyl-(alpha,beta)-methylene-diphosphonate. The Journal of Cell Biology, 128(1-2), 117-125. doi: 10.1083/jcb.128.1.117.

Hyman, A. A., Salser, S., Drechsel, D. N., Unwin, N., and Mitchison, T. J. (1992). Role of GTP hydrolysis in microtubule dynamics: Information from a slowly hydrolyzable analogue, GMPCPP. Molecular Biology of the Cell, 3(10), 1155-1167. doi: 10.1091/mbc.3.10.1155.

Ibarlucea-Benitez, I., Ferro, L. S., Drubin, D. G., and Barnes, G. (2018). Kinesins relocalize the chromosomal passenger complex to the midzone for spindle disassembly. The Journal of Cell Biology, 217(5), 1687-1700. doi: 10.1083/jcb.201708114.

Jeffery, J. M., Grigoriev, I., Poser, I., van der Horst, A., Hamilton, N., Waterhouse, N., Khanna, K. K. (2013). Centrobin regulates centrosome function in interphase cells by limiting pericentriolar matrix recruitment. Cell Cycle, 12(6), 899-906. doi: 10.4161/cc.23879.

Joukov, V., and De Nicolo, A. (2018). Aurora-PLK1 cascades as key signaling modules in the regulation of mitosis. Science Signaling, 11(543). doi: 10.1126/scisignal.aar4195.

Joukov, V., Walter, J. C., and De Nicolo, A. (2014). The Cep192-organized aurora A-Plk1 cascade is essential for centrosome cycle and bipolar spindle assembly. Molecular Cell, 55(4), 578-591. doi: 10.1016/j.molcel.2014.06.016.

Jungas, T., Perchey, R. T., Fawal, M., Callot, C., Froment, C., Burlet-Schiltz, O., Davy, A. (2016). Eph-mediated tyrosine phosphorylation of citron kinase controls abscission. The Journal of Cell Biology, 214(5), 555-569. doi: 10.1083/jcb.201602057.

Karthigeyan, D., Prasad, S. B., Shandilya, J., Agrawal, S., and Kundu, T. K. (2011). Biology of Aurora A kinase: Implications in cancer manifestation and therapy. Medicinal Research Reviews, 31(5), 757-793. doi: 10.1002/med.20203.

Katayama, H., Zhou, H., Li, Q., Tatsuka, M., and Sen, S. (2001). Interaction and feedback regulation between STK15/BTAK/Aurora-A kinase and protein phosphatase 1 through mitotic cell division cycle. The Journal of Biological Chemistry, 276(49), 46219-46224. doi:

Keck, J. M., Jones, M. H., Wong, C. C., Binkley, J., Chen, D., Jaspersen, S. L., Winey, M. (2011). A cell cycle phosphoproteome of the yeast centrosome. Science, 332(6037), 1557-1561. doi: 10.1126/science.1205193.

Keller, L. C., Wemmer, K. A., and Marshall, W. F. (2010). Influence of centriole number on mitotic spindle length and symmetry. Cytoskeleton, 67(8), 504-518. doi: 10.1002/cm.20462.

Khmelinskii, A., and Schiebel, E. (2008). Assembling the spindle midzone in the right place at the right time. Cell Cycle, 7(3), 283-286. doi: 10.4161/cc.7.3.5349.

Khodjakov, A., and Rieder, C. L. (1999). The sudden recruitment of gamma-tubulin to the centrosome at the onset of mitosis and its dynamic exchange throughout the cell cycle, do not require microtubules. The Journal of Cell Biology, 146(3), 585-596. doi: 10.1083/jcb.146.3.585.

Khodjakov, A., and Rieder, C. L. (2001). Centrosomes enhance the fidelity of cytokinesis in vertebrates and are required for cell cycle progression. The Journal of Cell Biology, 153(1), 237-242. doi: 10.1083/jcb.153.1.237.

Kim, J., Lee, K., and Rhee, K. (2015). PLK1 regulation of PCNT cleavage ensures fidelity of centriole separation during mitotic exit. Nature Communications, 6, 10076. doi: 10.1038/ncomms10076.

Kim, S., and Rhee, K. (2014). Importance of the CEP215-pericentrin interaction for centrosome maturation during mitosis. PloS One, 9(1), e87016. doi: 10.1371/journal.pone.0087016.

Kim, S., and Tsiokas, L. (2011). Cilia and cell cycle re-entry: More than a coincidence. Cell Cycle, 10(16), 2683-2690. doi: 10.4161/cc.10.16.17009.

.

Kirschner, M., and Mitchison, T. (1986). Beyond self-assembly: From microtubules to morphogenesis. Cell, 45(3), 329-342. doi: 10.1016/0092-8674(86)90318-1.

Kollman, J. M., Merdes, A., Mourey, L., and Agard, D. A. (2011). Microtubule nucleation by γ-tubulin complexes. Nature Reviews, Molecular Cell Biology, 12(11), 709-721. doi: 10.1038/nrm3209.

Korrodi-Gregório, L., Abrantes, J., Muller, T., Melo-Ferreira, J., Marcus, K., da Cruz e Silva, O. A., Esteves, P. J. (2013). Not so pseudo: The evolutionary history of protein phosphatase 1 regulatory subunit

2 and related pseudogenes. BMC Evolutionary Biology, 13, 242-2148-13-242. doi: 10.1186/1471-2148-13-242.

Korrodi-Gregório, L., Ferreira, M., Vintém, A. P., Wu, W., Muller, T., Marcus, K., da Cruz E Silva, E. F. (2013). Identification and characterization of two distinct PPP1R2 isoforms in human spermatozoa. BMC Cell Biology, 14(15), 15-2121-14-15. doi: 10.1186/1471-2121-14-15.

Krämer, A., Lukas, J., & Bartek, J. (2004). Checking out the centrosome. Cell Cycle, 3(11), 1390-1393. doi: 10.4161/cc.3.11.1252.

Kufer, T. A., Nigg, E. A., and Silljé, H. H. (2003). Regulation of Aurora-A kinase on the mitotic spindle. Chromosoma, 112(4), 159-163. doi: 10.1007/s00412-003-0265-1.

Kumar, S., Sharma, G., Chakraborty, C., Sharma, A. R., and Kim, J. (2017). Regulatory functional territory of PLK-1 and their substrates beyond mitosis. Oncotarget, 8(23), 37942-37962. doi: 10.18632/oncotarget.16290.

Kuriyama, R., and Borisy, G. G. (1981). Microtubule-nucleating activity of centrosomes in Chinese hamster ovary cells is independent of the centriole cycle but coupled to the mitotic cycle. The Journal of Cell Biology, 91(3 Pt 1), 822-826. doi: 10.1083/jcb.91.3.822.

Larsson, V. J., Jafferali, M. H., Vijayaraghavan, B., Figueroa, R. A., and Hallberg, E. (2018). Mitotic spindle assembly and γ-tubulin localisation depend on the integral nuclear membrane protein Samp1. Journal of Cell Science, 131(8), 1-12. doi: 10.1242/jcs.211664.

Lee, H. H., Elia, N., Ghirlando, R., Lippincott-Schwartz, J., & Hurley, J. H. (2008). Midbody targeting of the ESCRT machinery by a noncanonical coiled coil in CEP55. Science, 322(5901), 576-580. doi: 10.1126/science.1162042.

Lee, K., and Rhee, K. (2011). PLK1 phosphorylation of pericentrin initiates centrosome maturation at the onset of mitosis. The Journal of Cell Biology, 195(7), 1093-1101. doi: 10.1083/jcb.201106093.

Lee, S., and Rhee, K. (2010). CEP215 is involved in the dynein-dependent accumulation of pericentriolar matrix proteins for spindle pole formation. Cell Cycle, 9(4), 774-783. doi: 10.4161/cc.9.4.10667

Levine, M. S., Bakker, B., Boeckx, B., Moyett, J., Lu, J., Vitre, B., Holland, A. J. (2017). Centrosome amplification is sufficient to promote spontaneous tumorigenesis in mammals. Developmental Cell, 40(3), 313-322. doi: 10.1016/j.devcel.2016.12.022.

Li, M., Satinover, D. L., and Brautigan, D. L. (2007). Phosphorylation and functions of inhibitor-2 family of proteins. Biochemistry, 46(9), 2380-2389. doi: 10.1021/bi602369m.

Li, Q., Yan, X., Guo, L., Li, J., and Zang, Y. (2017). AMPK regulates anaphase central spindle length by phosphorylation of KIF4A. Journal of Molecular Cell Biology, 10(1), 2-17. doi: 10.1093/jmcb/mjx029

Lie-Jensen, A., Ivanauskiene, K., Malerød, L., Jain, A., Tan, K. W., Laerdahl, J. K., Haglund, K. (2019). Centralspindlin recruits ALIX to the midbody during cytokinetic abscission in *Drosophila* via a mechanism analogous to virus budding. Current Biology, 29(20), 3538-3548.e7. doi: 10.1016/j.cub.2019.09.025.

Lin, T. C., Neuner, A., Schlosser, Y. T., Scharf, A. N., Weber, L., and Schiebel, E. (2014). Cell-cycle dependent phosphorylation of yeast pericentrin regulates γ-TuSC-mediated microtubule nucleation. eLife, 3, e02208. doi: 10.7554/eLife.02208.

Lingle, W. L., Lukasiewicz, K., and Salisbury, J. L. (2005). Deregulation of the centrosome cycle and the origin of chromosomal instability in cancer. Advances in Experimental Medicine and Biology, 570, 393-421. doi: 10.1007/1-4020-3764-3_14.

Lioutas, A., and Vernos, I. (2013). Aurora A kinase and its substrate TACC3 are required for central spindle assembly. EMBO Reports, 14(9), 829-836. doi: 10.1038/embor.2013.109.

Liu Q, R. J. (2006). Aurora A, mitotic entry, and spindle bipolarity. Proceedings of the National Academy of Sciences of the United States of America, 103(15), 5811-5816. doi: 10.1073/pnas.0601425103.

Liu, P., Zupa, E., Neuner, A., Böhler, A., Loerke, J., Flemming, D., Schiebel, E. (2020). Insights into the assembly and activation of the microtubule nucleator γ-TuRC. Nature, 578(7795), 467-471. doi: 10.1038/s41586-019-1896-6.

Liu, Q., and Ruderman, J. V. (2006). Aurora A, mitotic entry, and spindle bipolarity. Proceedings of the National Academy of Sciences of the United States of America, 103(15), 5811-5816. doi: 10.1073/pnas.0601425103.

Liu, Z., and Weiner, O. D. (2016). Positioning the cleavage furrow: All you need is Rho. The Journal of Cell Biology, 213(6), 605-607. doi: 10.1083/jcb.201606010.

Lu, M. S., and Johnston, C. A. (2013). Molecular pathways regulating mitotic spindle orientation in animal cells. Development, 140(9), 1843-1856. doi: 10.1242/dev.087627.

Lüders, J. (2012). The amorphous pericentriolar cloud takes shape. Nature Cell Biology, 14(11), 1126-1128. doi: 10.1038/ncb2617.

Lukasiewicz, K. B., and Lingle, W. L. (2009). Aurora A, centrosome structure, and the centrosome cycle. Environmental and Molecular Mutagenesis, 50(8), 602-619. doi: 10.1002/em.20533.

Lutz, W., Lingle, W. L., McCormick, D., Greenwood, T. M., and Salisbury, J. L. (2001). Phosphorylation of centrin during the cell cycle and its role in centriole separation preceding centrosome duplication. The Journal of Biological Chemistry, 276(23), 20774-20780. doi: 10.1074/jbc.M101324200.

Maddox, P. S., Bloom, K. S., and Salmon, E. D. (2000). The polarity and dynamics of microtubule assembly in the budding yeast saccharomyces cerevisiae. Nature Cell Biology, 2(1), 36-41. doi: 10.1038/71357.

Magescas, J., Zonka, J. C., and Feldman, J. L. (2019). A two-step mechanism for the inactivation of microtubule organizing center function at the centrosome. eLife, 8, doi: 10.7554/eLife.47867.

Magnaghi-Jaulin, L., Eot-Houllier, G., Gallaud, E., and Giet, R. (2019). Aurora A protein kinase: To the centrosome and beyond. Biomolecules, 9(1), 28. doi: 10.3390/biom9010028.

Mangal S, Sacher J, Kim T, Osorio DS, Motegi F, Carvalho AX, Oegema K, Zanin E. (2018). TPXL-1 activates aurora A to clear contractile ring components from the polar cortex during cytokinesis. Journal of Cell Biology, 217(3), 837-848. doi: 10.1083/jcb.201706021.

Manning, J. A., Shalini, S., Risk, J. M., Day, C. L., and Kumar, S. (2010). A direct interaction with NEDD1 regulates gamma-tubulin recruitment to the centrosome. PloS One, 5(3). doi: 10.1371/journal.pone.0009618.

Margolin, G., Gregoretti, I. V., Cickovski, T. M., Li, C., Shi, W., Alber, M. S., and Goodson, H. V. (2012). The mechanisms of microtubule catastrophe and rescue: Implications from analysis of a dimer-scale computational model. Molecular Biology of the Cell, 23(4), 642-656. doi: 10.1091/mbc.E11-08-0688.

Margolis, R. L., and Wilson, L. (1998). Microtubule treadmilling: What goes around comes around. Bioassay: News and Reviews in Molecular, Cellular and Developmental Biology, 20(10), 830-836. doi: 10.1002/(SICI)1521-1878(199810)20:10<830::AID-BIES8>3.0.CO;2-N.

Marumoto, T., Honda, S., Hara, T., Nitta, M., Hirota, T., Kohmura, E., & Saya, H. (2003). Aurora-A kinase maintains the fidelity of early and late mitotic events in HeLa cells. The Journal of Biological Chemistry, 278(51), 51786-51795. doi: 10.1074/jbc.M306275200.

Mattison, C. P., and Winey, M. (2006). The centrosome cycle. Results and Problems in Cell Differentiation, Philipp Kaldis (editor), Springer, 42, 111-146. doi: 10.1007/b136685.

Mbom, B. C., Nelson, W. J., and Barth, A. (2013). β-Catenin at the centrosome: Discrete pools of β-catenin communicate during mitosis and may co-ordinate centrosome functions and cell cycle progression. BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology, 35(9), 804-809. doi: 10.1002/bies.201300045.

McCollum, D. (2004). Cytokinesis: The central spindle takes center stage. Current Biology, 14(22), R953-5. doi: 10.1016/j.cub.2004.10.040.

McDonald, B., and Martin-Serrano, J. (2009). No strings attached: The ESCRT machinery in viral budding and cytokinesis. Journal of Cell Science, 122(Pt 13), 2167-2177. doi: 10.1242/jcs.028308.

McKenzie, C., Bassi, Z. I., Debski, J., Gottardo, M., Callaini, G., Dadlez, M., and D'Avino, P. P. (2016). Cross-regulation between Aurora B and citron kinase controls midbody architecture in cytokinesis. Open Biology, 6(3), 1-15. doi: 10.1098/rsob.160019.

Meadows, J. C. (2013). Interplay between mitotic kinesins and the Aurora kinase-PP1 (protein phosphatase 1) axis. Biochemical Society Transactions, 41(6), 1761-1765. doi: 10.1042/BST20130191.

Melki, R., Carlier, M. F., Pantaloni, D., and Timasheff, S. N. (1989). Cold depolymerization of microtubules to double rings: Geometric stabilization of assemblies. Biochemistry, 28(23), 9143-9152. doi: 10.1021/bi00449a028.

Meng, L., Park, J. E., Kim, T. S., Lee, E. H., Park, S. Y., Zhou, M., Lee, K. S. (2015). Bimodal interaction of mammalian polo-like kinase 1 and a centrosomal scaffold, Cep192, in the regulation of bipolar spindle formation. Molecular and Cellular Biology, 35(15), 2626-2640. doi: 10.1128/MCB.00068-15.

Meraldi, P. (2016). Centrosomes in spindle organization and chromosome segregation: A mechanistic view. Chromosome Research: An International Journal on the Molecular, Supramolecular and Evolutionary Aspects of Chromosome Biology, 24(1), 19-34. doi: 10.1007/s10577-015-9508-2.

Meraldi, P., Honda, R., and Nigg, E. A. (2002). Aurora-A overexpression reveals tetraploidization as a major route to centrosome amplification in p53-/- cells. The EMBO Journal, 21(4), 483-492. doi: 10.1093/emboj/21.4.483.

Meraldi, P., and Nigg, E. A. (2001). Centrosome cohesion is regulated by a balance of kinase and phosphatase activities. Journal of Cell Science, 114(Pt 20), 3749-3757.

Meraldi, P., and Nigg, E. A. (2002). The centrosome cycle. FEBS Letters, 521(1-3), 9-13. doi: 10.1007/b136685.

Mi, J., Guo, C., Brautigan, D. L., and Larner, J. M. (2007). Protein phosphatase-1alpha regulates centrosome splitting through Nek2. Cancer Research, 67(3), 1082-1089. doi: 10.1158/0008-5472.CAN-06-3071.

Mitchison, T. J. (1993). Localization of an exchangeable GTP binding site at the plus end of microtubules. Science, 261(5124), 1044-1047. doi: 10.1126/science.8102497.

Miyamoto, T., Akutsu, S. N., Fukumitsu, A., Morino, H., Masatsuna, Y., Hosoba, K., Matsuura, S. (2017). PLK1-mediated phosphorylation of WDR62/MCPH2 ensures proper mitotic spindle orientation. Human Molecular Genetics, 26(22), 4429-4440. doi: 10.1093/hmg/ddx330.

Morris, E. J., Nader, G. P., Ramalingam, N., Bartolini, F., and Gundersen, G. G. (2014). Kif4 interacts with EB1 and stabilizes microtubules downstream of rho-mDia in migrating fibroblasts. PloS One, 9(3). doi: 10.1371/journal.pone.0091568.

Moura, M., and Conde, C. (2019). Phosphatases in mitosis: Roles and regulation. Biomolecules, 9(2), 55. doi: 10.3390/biom9020055.

Mukherjee, M., Sabir, S., O'Regan, L., Sampson, J., Richards, M. W., Huguenin-Dezot, N., Bayliss, R. (2018). Mitotic phosphorylation regulates Hsp72 spindle localization by uncoupling ATP binding from substrate release. Science Signaling, 11(543). 1-27. doi: 10.1126/scisignal.aao2464.

Müller-Reichert, T., Chrétien, D., Severin, F., and Hyman, A. A. (1998). Structural changes at microtubule ends accompanying GTP hydrolysis: Information from a slowly hydrolyzable analogue of GTP, guanylyl (alpha,beta)methylenediphosphonate. Proceedings of the National Academy of Sciences of the United States of America, 95(7), 3661-3666. doi: 10.1073/pnas.95.7.3661.

Nähse, V., Christ, L., Stenmark, H., and Campsteijn, C. (2017). The abscission checkpoint: Making it to the final cut. Trends in Cell Biology, 27(1), 1-11. doi: 10.1016/j.tcb.2016.10.001

Nasa, I., Trinkle-Mulcahy, L., Douglas, P., Chaudhuri, S., Lees-Miller, S. P., Lee, K. S., and Moorhead, G. B. (2017). Recruitment of PP1 to the centrosomal scaffold protein CEP192. Biochemical and Biophysical Research Communications, 484(4), 864-870. doi: 10.1016/j.bbrc.2017.02.004.

Naso, F. D., Sterbini, V., Crecca, E., Asteriti, I. A., Russo, A. D., Giubettini, M., Guarguaglini, G. (2020). Excess TPX2 interferes with microtubule disassembly and nuclei reformation at mitotic exit. Cells, 9(2), 374. doi: 10.3390/cells9020374.

Nigg, E. A., and Stearns, T. (2011). The centrosome cycle: Centriole biogenesis, duplication and inherent asymmetries. Nature Cell Biology, 13(10), 1154-1160. doi: 10.1038/ncb2345.

Nikonova, A. S., Astsaturov, I., Serebriiskii, I. G., Dunbrack, R. L.,Jr, and Golemis, E. A. (2013). Aurora A kinase (AURKA) in normal and pathological cell division. Cellular and Molecular Life Sciences: 70(4), 661-687. doi: 10.1007/s00018-012-1073-7.

Nogales, E., Downing, K. H., Amos, L. A., and Löwe, J. (1998). Tubulin and FtsZ form a distinct family of GTPases. Nature Structural Biology, 5(6), 451-458. doi: 10.1038/nsb0698-451.

Nunes Bastos, R., Gandhi, S. R., Baron, R. D., Gruneberg, U., Nigg, E. A., and Barr, F. A. (2013). Aurora B suppresses microtubule dynamics and limits central spindle size by locally activating KIF4A. The Journal of Cell Biology, 202(4), 605-621. doi: 10.1083/jcb.201301094.

Oakley, B. R., Paolillo, V., and Zheng, Y. (2015). gamma-Tubulin complexes in microtubule nucleation and beyond. Molecular Biology of the Cell, 26(17), 2957-2962. 10.1091/mbc.E14-11-1514. doi: 10.1091/mbc.E14-11-1514.

Ohashi, A., Ohori, M., and Iwai, K. (2016). Motor activity of centromere-associated protein-E contributes to its localization at the center of the midbody to regulate cytokinetic abscission. Oncotarget, 7(48), 79964-79980. doi: 10.18632/oncotarget.13206

Palazzo, A. F., Cook, T. A., Alberts, A. S., and Gundersen, G. G. (2001). mDia mediates rho-regulated formation and orientation of stable microtubules. Nature Cell Biology, 3(8), 723-729. doi: 10.1038/35087035.

Palazzo, R. E., Vogel, J. M., Schnackenberg, B. J., Hull, D. R., and Wu, X. (2000). Centrosome maturation. Current Topics in Developmental Biology, 49, 449-470. doi: 10.1016/s0070-2153(99)49021-0.

Pamula, M. C., Carlini, L., Forth, S., Verma, P., Suresh, S., Legant, W. R., Kapoor, T. M. (2019). High-resolution imaging reveals how the spindle midzone impacts chromosome movement. The Journal of Cell Biology, 218(8), 2529-2544. doi: 10.1083/jcb.201904169.

Patharkar, O. R., and Walker, J. C. (2018). Advances in abscission signaling. Journal of Experimental Botany, 69(4), 733-740. doi: 10.1093/jxb/erx256.

Patzke, S., Hauge, H., Sioud, M., Finne, E. F., Sivertsen, E. A., Delabie, J., Aasheim, H. C. (2005). Identification of a novel centrosome/microtubule-associated coiled-coil protein involved in cell-cycle progression and spindle organization. Oncogene, 24(7), 1159-1173. doi: 10.1038/sj.onc.1208267.

Peel, N., Iyer, J., Naik, A., Dougherty, M. P., Decker, M., and O'Connell, K. F. (2017). Protein phosphatase 1 down regulates ZYG-1 levels to limit centriole duplication. PLoS Genetics, 13(1), 1-25. .doi: 10.1371/journal.pgen.1006543.

Perchey, R. T., Serres, M. P., Nowosad, A., Creff, J., Callot, C., Gay, A., Besson, A. (2018). p27(Kip1) regulates the microtubule bundling activity of PRC1. Biochimica Et Biophysica Acta.Molecular Cell Research, 1865(11 Pt A), 1630-1639. doi: 10.1016/j.bbamcr.2018.08.010.

Petretti, C., Savoian, M., Montembault, E., Glover, D. M., Prigent, C., and Giet, R. (2006). The PITSLRE/CDK11p58 protein kinase promotes centrosome maturation and bipolar spindle formation. EMBO Reports, 7(4), 418-424. doi: 10.1038/sj.embor.7400639.

Petronczki, M., Lénárt, P., and Peters, J. M. (2008). Polo on the rise-from mitotic entry to cytokinesis with Plk1. Developmental Cell, 14(5), 646-659. doi: 10.1016/j.devcel.2008.04.014.

Piehl, M., Tulu, U. S., Wadsworth, P., and Cassimeris, L. (2004). Centrosome maturation: Measurement of microtubule nucleation throughout the cell cycle by using GFP-tagged EB1. Proceedings of the National Academy of Sciences of the United States of America, 101(6), 1584-1588. doi: 10.1073/pnas.0308205100.

Pike, T., Brownlow, N., Kjaer, S., Carlton, J., and Parker, P. J. (2016). PKCε switches Aurora B specificity to exit the abscission checkpoint. Nature Communications, 7, 13853. doi: 10.1038/ncomms13853.

Pinyol, R., Scrofani, J., & Vernos, I. (2013). The role of NEDD1 phosphorylation by Aurora A in chromosomal microtubule nucleation and spindle function. Current Biology, 23(2), 143-149. doi: 10.1016/j.cub.2012.11.046.

Plotnikova, O. V., Pugacheva, E. N., and Golemis, E. A. (2009). Primary cilia and the cell cycle. Methods in Cell Biology, Roger D. Sloboda (editor), ScienceDirect. 94, 137-160. doi: 10.1016/S0091-679X(08)94007-3.

Pohl, C. (2017). The midbody and its remnant in cell polarization and asymmetric cell division. Results and Problems in Cell Differentiation, 61, 165-182. doi: 10.1007/978-3-319-53150-2_7.

Prosser, S. L., and Pelletier, L. (2017). Mitotic spindle assembly in animal cells: A fine balancing act. Nature Reviews. Molecular Cell Biology, 18(3), 187-201. doi: 10.1038/nrm.2016.162.

Puntoni, F., and Villa-Moruzzi, E. (1995). Phosphorylation of the inhibitor-2 of protein phosphatase-1 by cdc2-cyclin B and GSK3. Biochemical and Biophysical Research Communications, 207(2), 732-739. doi: 10.1006/bbrc.1995.1248.

Raynaud-Messina, B., and Merdes, A. (2007). Gamma-tubulin complexes and microtubule organization. Current Opinion in Cell Biology, 19(1), 24-30. doi: 10.1016/j.ceb.2006.12.008.

Rivera-Rivera, Y., and Saavedra, H. I. (2016). Centrosome - a promising anti-cancer target. Biologics: Targets and Therapy, 10, 167-176. doi: 10.2147/BTT.S87396.

Rodionov, V. I., and Borisy, G. G. (1997). Microtubule treadmilling in vivo. Science, 275(5297), 215-218. doi: 10.1126/science.275.5297.215.

Rovina, D., Fontana, L., Monti, L., Novielli, C., Panini, N., Sirchia, S. M., Larizza, L. (2014). Microtubule-associated protein/microtubule affinity-regulating kinase 4 (MARK4) plays a role in cell cycle progression and cytoskeletal dynamics. European Journal of Cell Biology, 93(8-9), 355-365. doi: 10.1016/j.ejcb.2014.07.004.

Ruchaud, S., Carmena, M., and Earnshaw, W. C. (2007). Chromosomal passengers: Conducting cell division. Nature Reviews. Molecular Cell Biology, 8(10), 798-812. doi: 10.1038/nrm2257.

Sakashita, G., Shima, H., Komatsu, M., Urano, T., Kikuchi, A., and Kikuchi, K. (2003). Regulation of type 1 protein phosphatase/inhibitor-2 complex by glycogen synthase kinase-3beta in intact cells. Journal of Biochemistry, 133(2), 165-171. doi: 10.1093/jb/mvg020.

Satinover, D. L., Brautigan, D. L., and Stukenberg, P. T. (2006). Aurora-A kinase and inhibitor-2 regulate the cyclin threshold for mitotic entry in Xenopus early embryonic cell cycles. Cell Cycle, 5(19), 2268-2274. doi: 10.4161/cc.5.19.3316.

Satinover, D. L., Leach, C. A., Stukenberg, P. T., and Brautigan, D. L. (2004). Activation of Aurora-A kinase by protein phosphatase inhibitor-2, a bifunctional signaling protein. Proceedings of the National Academy of Sciences of the United States of America, 101(23), 8625-8630. doi: 10.1073/pnas.0402966101.

Schatten, H. (2008). The mammalian centrosome and its functional significance. Histochemistry and Cell Biology, 129(6), 667-686. doi: 10.1007/s00418-008-0427-6.

Schatten, H., and Sun, Q. Y. (2018). Functions and dysfunctions of the mammalian centrosome in health, disorders, disease, and aging. Histochemistry and Cell Biology, 150(4), 303-325. doi: 10.1007/s00418-018-1698-1.

Schuldt, A. (2010). Cytoskeleton: Midzone microtubule management. Nature Reviews. Molecular Cell Biology, 11(9), 602-603. doi: 10.1038/nrm2965.

Shi, X., Sun, X., Liu, M., Li, D., Aneja, R., and Zhou, J. (2011). CEP70 protein interacts with γ-tubulin to localize at the centrosome and is critical for mitotic spindle assembly. The Journal of Biological Chemistry, 286(38), 33401-33408. doi: 10.1074/jbc.M111.252262.

Siegel, R. L., Miller, K. D., & Jemal, A. (2020). Cancer statistics, (2020). CA: A Cancer Journal for Clinicians, 70(1), 7-30. doi: 10.3322/canjclin.57.1.43.

Sillibourne, J. E., Hurbain, I., Grand-Perret, T., Goud, B., Tran, P., and Bornens, M. (2013). Primary ciliogenesis requires the distal appendage component Cep123. Biology Open, 2(6), 535-545. doi: 10.1242/bio.20134457.

Sluder, G. (2005). Two-way traffic: Centrosomes and the cell cycle. Nature Reviews. Molecular Cell Biology, 6(9), 743-748. doi: 10.1038/nrm1712.

Sorokin, S. P. (1968). Reconstructions of centriole formation and ciliogenesis in mammalian lungs. Journal of Cell Science, 3(2), 207-230.

Spiegelman, B. M., Penningroth, S. M., and Kirschner, M. W. (1977). Turnover of tubulin and the N site GTP in Chinese hamster ovary cells. Cell, 12(3), 587-600. doi: 10.1016/0092-8674(77)90259-8.

Srsen, V., Gnadt, N., Dammermann, A., and Merdes, A. (2006). Inhibition of centrosome protein assembly leads to p53-dependent exit from the cell cycle. The Journal of Cell Biology, 174(5), 625-630. doi: 10.1083/jcb.20060605.

Steigemann, P., and Gerlich, D. W. (2009). Cytokinetic abscission: Cellular dynamics at the midbody. Trends in Cell Biology, 19(11), 606-616. doi: 10.1016/j.tcb.2009.07.008.

Steigemann, P., Wurzenberger, C., Schmitz, M. H., Held, M., Guizetti, J., Maar, S., and Gerlich, D. W. (2009). Aurora B-mediated abscission checkpoint protects against tetraploidization. Cell, 136(3), 473-484. doi: 10.1016/j.cell.2008.12.020.

Subramanian, R., Wilson-Kubalek, E. M., Arthur, C. P., Bick, M. J., Campbell, E. A., Darst, S. A., Kapoor, T. M. (2010). Insights into antiparallel microtubule crosslinking by PRC1, a conserved nonmotor microtubule binding protein. Cell, 142(3), 433-443. doi: 10.1016/j.cell.2010.07.012.

Sun, S., Sun, L., Zhou, X., Wu, C., Wang, R., Lin, S. H., and Kuang, J. (2016). Phosphorylation-dependent activation of the ESCRT function of ALIX in cytokinetic abscission and retroviral budding. Developmental Cell, 36(3), 331-343. doi: 10.1016/j.devcel.2016.01.001.

Takahashi, M., Yamagiwa, A., Nishimura, T., Mukai, H., and Ono, Y. (2002). Centrosomal proteins CG-NAP and kendrin provide microtubule nucleation sites by anchoring gamma-tubulin ring complex. Molecular Biology of the Cell, 13(9), 3235-3245. doi: 10.1091/mbc.e02-02-0112.

Takaoka, M., Saito, H., Takenaka, K., Miki, Y., and Nakanishi, A. (2014). BRCA2 phosphorylated by PLK1 moves to the midbody to regulate cytokinesis mediated by nonmuscle myosin IIC. Cancer Research, 74(5), 1518-1528. doi: 10.1158/0008-5472.CAN-13-0504.

Tian, G., Bhamidipati, A., Cowan, N. J., and Lewis, S. A. (1999). Tubulin folding cofactors as GTPase-activating proteins. GTP hydrolysis and the assembly of the alpha/beta-tubulin heterodimer. The Journal of Biological Chemistry, 274(34), 24054-24058. doi: 10.1074/jbc.274.34.24054.

Tovey, C. A., and Conduit, P. T. (2018). Microtubule nucleation by γ-tubulin complexes and beyond. Essays in Biochemistry, 62(6), 765-780. doi: 10.1042/EBC20180028.

Uehara, R., Tsukada, Y., Kamasaki, T., Poser, I., Yoda, K., Gerlich, D. W., and Goshima, G. (2013). Aurora B and Kif2A control microtubule length for assembly of a functional central spindle during anaphase. The Journal of Cell Biology, 202(4), 623-636. doi: 10.1083/jcb.201302123.

van der Waal, M. S., Hengeveld, R. C., van der Horst, A., and Lens, S. M. (2012). Cell division control by the chromosomal passenger complex. Experimental Cell Research, 318(12), 1407-1420. doi: 10.1016/j.yexcr.2012.03.015.

van Heesbeen, R. G. H. P., Raaijmakers, J. A., Tanenbaum, M. E., Halim, V. A., Lelieveld, D., Lieftink, C., Medema, R. H. (2017). Aurora A, MCAK, and Kif18b promote Eg5-independent spindle formation. Chromosoma, 126(4), 473-486. doi: 10.1007/s00412-016-0607-4.

Vandré, D. D., Feng, Y., and Ding, M. (2000). Cell cycle-dependent phosphorylation of centrosomes: Localization of phosphopeptide specific antibodies to the centrosome. Microscopy Research and Technique, 49(5), 458-466. doi: 10.1002/(SICI)1097-0029(20000601)49:5<458::AID-JEMT8>3.0.CO;2-#.

Vemu, A., Szczesna, E., Zehr, E. A., Spector, J. O., Grigorieff, N., Deaconescu, A. M., and Roll-Mecak, A. (2018). Severing enzymes amplify microtubule arrays through lattice GTP-tubulin incorporation. Science, 361(6404). doi: 10.1126/science.aau1504.

Venoux, M., Basbous, J., Berthenet, C., Prigent, C., Fernandez, A., Lamb, N. J., and Rouquier, S. (2008). ASAP is a novel substrate of the oncogenic mitotic kinase Aurora-A: Phosphorylation on Ser625 is essential to spindle formation and mitosis. Human Molecular Genetics, 17(2), 215-224. doi: 10.1093/hmg/ddm298.

VerPlank, L., and Li, R. (2005). Cell cycle-regulated trafficking of Chs2 controls actomyosin ring stability during cytokinesis. Molecular Biology of the Cell, 16(5), 2529-2543. doi: 10.1091/mbc.e04-12-1090.

Vietri, M., Schink, K. O., Campsteijn, C., Wegner, C. S., Schultz, S. W., Christ, L., Stenmark, H. (2015). Spastin and ESCRT-III coordinate mitotic spindle disassembly and nuclear envelope sealing. Nature, 522(7555), 231-235. doi: 10.1038/nature14408.

Vora, S., and Phillips, B. T. (2015). Centrosome-associated degradation limits β-catenin inheritance by daughter cells after asymmetric division. Current Biology, 25(8), 1005-1016. doi: 10.1016/j.cub.2015.02.020.

Wade, R. H. (2007). Microtubules: An overview. Methods in Molecular Medicine, Jun Zhou (editor), Humana Press. 137, 1-16. doi: 10.2119/molecular%20medicine-2006-00038.

Walczak, C. E., and Shaw, S. L. (2010). A MAP for bundling microtubules. Cell, 142(3), 364-367. doi: 10.1016/j.cell.2010.07.023.

Wang, G., Jiang, Q., and Zhang, C. (2014). The role of mitotic kinases in coupling the centrosome cycle with the assembly of the mitotic spindle. Journal of Cell Science, 127(Pt 19), 4111-4122. doi: 10.1242/jcs.151753.

Wang W, Stukenberg PT, Brautigan DL. (2008). Phosphatase inhibitor-2 balances protein phosphatase 1 and aurora B kinase for chromosome segregation and cytokinesis in human retinal epithelial cells. Molecular Biology of the Cell, 19(11), 4852-4862. doi: 10.1091/mbc.e08-05-0460.

Waterman-Storer, C. M., and Salmon, E. D. (1997). Microtubule dynamics: Treadmilling comes around again. Current Biology, 7(6), R369-72. doi: 10.1016/s0960-9822(06)00177-1.

Wei, J. H., Zhang, Z. C., Wynn, R. M., and Seemann, J. (2015). GM130 regulates Golgi-derived spindle assembly by activating TPX2 and capturing microtubules. Cell, 162(2), 287-299. doi: 10.1016/j.cell.2015.06.014.

White, E. A., and Glotzer, M. (2012). Centralspindlin: At the heart of cytokinesis. Cytoskeleton, 69(11), 882-892. doi: 10.1002/cm.21065.

Wilde, A., Lizarraga, S. B., Zhang, L., Wiese, C., Gliksman, N. R., Walczak, C. E., and Zheng, Y. (2001). Ran stimulates spindle assembly by altering microtubule dynamics and the balance of motor activities. Nature Cell Biology, 3(3), 221-227. doi: 10.1038/35060000.

Winey, M., and Bloom, K. (2012). Mitotic spindle form and function. Genetics, 190(4), 1197-1224. doi: 10.1534/genetics.111.128710.

Winey, M., and O'Toole, E. (2014). Centriole structure. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 369(1650), 1-9. doi: 10.1098/rstb.2013.0457.

Woodruff, J. B., Drubin, D. G., and Barnes, G. (2010). Mitotic spindle disassembly occurs via distinct subprocesses driven by the anaphase-promoting complex, Aurora B kinase, and kinesin-8. The Journal of Cell Biology, 191(4), 795-808. doi: 10.1083/jcb.201006028.

Woodruff, J. B., Wueseke, O., and Hyman, A. A. (2014). Pericentriolar material structure and dynamics. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 369(1650), 20130459. doi: 10.1098/rstb.2013.0459.

Woodruff, J. B., Wueseke, O., Viscardi, V., Mahamid, J., Ochoa, S. D., Bunkenborg, J., Widlund, P. O., Pozniakovsky, A., Zanin, E., Bahmanyar, S., Zinke, A., Hong, S. H., Decker, M., Baumeister, W., Andersen, J. S., Oegema, K., Hyman, A. A. (2015). Centrosomes. regulated assembly of a supramolecular centrosome scaffold in vitro. Science, 348(6236), 808-812. doi: 10.1126/science.aaa3923.

Yaguchi, K., Yamamoto, T., Matsui, R., Tsukada, Y., Shibanuma, A., Kamimura, K., Uehara, R. (2018). Uncoordinated centrosome cycle underlies the instability of non-diploid somatic cells in mammals. The Journal of Cell Biology, 217(7), 2463-2483. doi: 10.1083/jcb.201701151.

Ye, A. A., Torabi, J., and Maresca, T. J. (2016). Aurora A kinase amplifies a midzone phosphorylation gradient to promote high-fidelity cytokinesis. The Biological Bulletin, 231(1), 61-72. doi:10.1086/689591.

Yenjerla, M., Lopus, M., and Wilson, L. (2010). Analysis of dynamic instability of steady-state microtubules in vitro by video-enhanced differential interference contrast microscopy with an appendix by Emin Oroudjev. Methods in Cell Biology, Leslie Wilson, John J. Correia (Editors), ScienceDirect, 95, 189-206. doi: 10.1016/S0091-679X(10)95011-5.

Yu, C. H., Redemann, S., Wu, H. Y., Kiewisz, R., Yoo, T. Y., Conway, W., Needleman, D. (2019). Central-spindle microtubules are strongly coupled to chromosomes during both anaphase A and anaphase B. Molecular Biology of the Cell, 30(19), 2503-2514. doi: 10.1091/mbc.E19-01-0074.

Zhang, X., Chen, Q., Feng, J., Hou, J., Yang, F., Liu, J., Zhang, C. (2009). Sequential phosphorylation of Nedd1 by Cdk1 and Plk1 is required for targeting of the gammaTuRC to the centrosome. Journal of Cell Science, 122(Pt 13), 2240-2251. doi: 10.1242/jcs.042747.

Zhao, W. M., Seki, A., & Fang, G. (2006). Cep55, a microtubule-bundling protein, associates with centralspindlin to control the midbody integrity and cell abscission during cytokinesis. Molecular Biology of the Cell, 17(9), 3881-3896. doi: 10.1091/mbc.e06-01-0015.

Zhou H, Kuang J, Zhong L, Kuo WL, Gray JW, Sahin A, Brinkley BR, Sen S. (1998). Tumour amplified kinase STK15/BTAK induces centrosome amplification, aneuploidy and transformation. Nature Genetics, 20(2), 189-193. doi: 10.1038/2496.

Zhu, C., and Jiang, W. (2005). Cell cycle-dependent translocation of PRC1 on the spindle by Kif4 is essential for midzone formation and cytokinesis. Proceedings of the National Academy of Sciences of the United States of America, 102(2), 343-348. doi: 10.1073/pnas.0408438102.

Zimmerman, W. C., Sillibourne, J., Rosa, J., and Doxsey, S. J. (2004). Mitosis-specific anchoring of gamma tubulin complexes by pericentrin controls spindle organization and mitotic entry. Molecular Biology of the Cell, 15(8), 3642-3657. doi: 10.1091/mbc.e03-11-0796.

Zorba, A., Buosi, V., Kutter, S., Kern, N., Pontiggia, F., Cho, Y. J., and Kern, D. (2014). Molecular mechanism of aurora A kinase autophosphorylation and its allosteric activation by TPX2. eLife, 3, e02667. doi: 10.7554/eLife.02667.